Using urinary MCP-1 and TWEAK to assess disease activity in a cohort of South African patients with lupus nephritis

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DECLARATION

I, Jody Alan Rusch, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce, for the purpose of research, either the whole or any portion of the contents in any manner whatsoever.

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LIST OF TABLES AND FIGURE

GLOSSARY OF TERMS

ALUGEN Registry

- The African Lupus Genetics Network Registry is a multi-centred framework seeking to prospectively assess outcomes in SLE patients in Africa

Chemokine

- Chemokines are a family of chemoattractant cytokines (small proteins secreted by cells that influence the immune system) which play a vital role in cell migration through blood vessels from blood into tissue and vice versa, and in the induction of cell movement in response to a chemical (chemokine) gradient by a process known as chemotaxis

Cytokine

- A group of small proteins that are important in cell signalling. They are released by cells and affect the behaviour of other cells

ELISA

- The enzyme-linked immunosorbent assay is a common laboratory technique which is used to measure the concentration of an analyte (usually antibodies or antigens) in solution

Lupus Nephritis (LN)

- A common and serious complication in patients with SLE, whereby there is inflammation of the kidneys

Monocyte Chemoattractant protein-1 (MCP-1)

- A leucocyte chemotactic factor involved in mediating renal inflammation and injury in LN

Systemic Lupus Erythematosus (SLE)

- A multi-systemic autoimmune disease

SLEDAI-2K Score

O A scoring system to assess Systemic Lupus Erythematosus disease activity

Renal SLEDAI-2K Score

- A scoring system to assess kidney disease activity in patients with LN and consists of four parameters (haematuria, pyuria, proteinuria and urinary casts)

SLICC/ACR Damage Index

- A scoring system used to assess disease activity and damage in SLE patients.

Tumour Necrosis Factor-like Weak Inducer of Apoptosis (TWEAK)

- A cytokine and member of the tumour necrosis factor superfamily, TWEAK is involved in pro-inflammatory responses.

LIST OF ABBREVIATIONS

ANA	Anti-Nuclear Antibody
Anti-dsDNA	Anti-Double Stranded Deoxyribonucleic Acid
Anti-Sm	Anti-Smith
C3	Complement Component 3
C4	Complement Component 4
CC	Chemotactic cytokine
CCR2	Chemokine Receptor 2
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-Linked Immunosorbent Assay
ESRD	End-Stage Renal Disease
FBC	Full Blood Count
Fn14	Fibroblast Growth Factor Inducible 14
GSH	Groote Schuur Hospital
HIVAN	Human Immunodeficiency Virus Associated Nephropathy
HREC	Human Research Ethics Committee
ICAM-1	Intercellular Adhesion Molecule-1
IP-10	Interferon gamma induced Protein-10
ISN/RPS	International Society of Nephrology/ Renal Pathology Society
LN	Lupus Nephritis
MCP-1	Monocyte Chemoattractant Protein-1
MCP-1/Cr	Urinary MCP-1 normalised to urinary creatinine
MDRD	Modification of Diet in Renal Disease
MIP-1á	Macrophage Inflammatory Protein-1á
MMP-1	Matrix Metalloproteinase-1
MMP-9	Matrix Metalloproteinase-9

RANTES	Regulated in Activation, Normal T Expressed and Secreted
SLE	Systemic Lupus Erythematosus
SLEDAI-2K Score	SLE Disease Activity Index 2000
SLICC/ACR Damage Index	Systemic Lupus Collaborating Clinics/American College of Rheumatology Damage Index
TNF	Tumour Necrosis Factor
TWEAK	Tumour Necrosis Factor-like Weak Inducer of Apoptosis
TWEAK/Cr	Urinary TWEAK normalised to urinary creatinine
uMCP-1	Urinary MCP-1
UPCR	Urine Protein to Creatinine Ratio
uTWEAK	Urinary TWEAK
VCAM-1	Vascular Adhesion Molecule-1

ABSTRACT

Background:

Renal involvement is common in systemic lupus erythematosus (SLE) and can lead to chronic kidney disease (CKD). Diagnosis of lupus nephritis (LN) is dependent on renal biopsy. Due to its invasiveness, repeat renal biopsy for monitoring disease activity is not recommended, thus creating a need for non-invasive and accurate biomarkers. Monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-like weak inducer of apoptosis (TWEAK) have been implicated in the pathogenesis of LN and are thus potential biomarkers for disease activity monitoring.

Methods:

In this study urinary MCP-1 (uMCP-1) and TWEAK (uTWEAK), together with standard markers of disease activity, were analysed in a cohort of 50 biopsy-proven LN patients at baseline, after six-months of induction therapy, and at one-year.

Results:

Throughout the study there was correlation between uMCP-1 and uTWEAK ($r=0.52$, $p<0.001$). Both biomarkers also correlated with standard of care tests and clinical scores. The median [interquartile range] of uMCP-1 and uTWEAK were significantly increased in the active group when compared to the quiescent group (1440 [683–2729] vs 256 [175–477] pg/mL, $p<0.0001$, and 209 [117–312] vs 74 [11–173] pg/mL, $p=0.0008$, respectively). After completion of induction therapy in the active group, there was no significant difference in biomarker results between the groups. The sensitivity and specificity for indicating disease activity was 95% and 73% for uMCP-1 (area under curve [AUC]=0.875), and 60% and 90% for uTWEAK (AUC=0.783), respectively.

Conclusion:

uMCP-1 and uTWEAK reflect LN disease activity, and correlate with standard of care biomarkers in a South African cohort. Further studies are needed to assess additional clinical benefit.

Keywords:

MCP-1, monocyte chemoattractant protein-1; TWEAK, tumour necrosis factor-like weak inducer of apoptosis; urinary biomarkers; lupus nephritis; systemic lupus erythematosus; disease activity; renal biopsy

(WORD COUNT – 257)

CHAPTER ONE

CHAPTER 1: INTRODUCTION & LITERATURE REVIEW

1.1 Aim & Objectives

1.1.1 Aim

The aim of this study was to prospectively assess the diagnostic performance and value of the urinary biomarkers MCP-1 and TWEAK as predictors of disease activity status in LN

1.1.2 Objectives

The objectives of this study were as follows:

1. To determine the participants disease activity level at baseline using currently available modalities including:
    a. Biochemical parameters: urinalysis, serum creatinine, complements (C3 and C4) and autoantibodies (anti-nuclear antibody [ANA], anti-double stranded deoxyribonucleic acid [anti-dsDNA] and anti-Smith [anti-Sm]).
    b. Disease activity scores: Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), renal SLEDAI (rSLEDAI) and Systemic Lupus Collaborating Clinics/American College of Rheumatology Damage Index (SLICC) scores
    c. Renal biopsy – only the active group had a new biopsy
2. To analyse the uMCP-1 and uTWEAK levels of the participants using ELISA methodology
3. To determine the utility of uMCP-1 and uTWEAK in the assessment of disease activity status in LN

1.2 Introduction

1.2.1 Background

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease characterised by complex protean clinical manifestations involving multiple organ systems. The patients' presentation and course of the disease are highly variable, relapsing and remitting, and ranging from indolent to fulminant. Their prognosis is also variable and depends upon the organ systems involved. Although considerable advances have been made in understanding the complex clinical features and unpredictable progression of SLE, the pathogenesis remains unclear. The features of SLE are related to immune dysregulation, including autoantibody production, activation of complement pathways, and immune complex deposition in target tissues(1). Recent investigations have addressed the roles of chemokines and cytokines in the regulation of lupus disease activity and specific organ involvement, including the kidneys(2,3).

Renal involvement is common in SLE, with approximately 60% of patients developing clinically significant renal disease(4). Most renal abnormalities emerge soon after diagnosis, commonly within the first six to 36 months(4). A wide range of abnormalities have been described in lupus nephritis (LN), from asymptomatic proteinuria or microscopic haematuria with normal renal function, to severe nephrotic syndrome or acute renal failure(5). Some patients with LN develop hypertension(6). Approximately 10–15% of nephritis patients progress to end-stage renal disease (ESRD) requiring haemodialysis(4). An abnormal urinalysis (protein or blood) or an elevation in serum creatinine should alert the clinician to the possible presence of LN. Unfortunately, significant kidney damage usually occurs before renal impairment is detected by laboratory tests.

LN is an immune complex glomerulonephritis involving multiple factors which include genetic predisposition, epigenetic modification and environmental components. The pathogenesis of LN is complex involving both innate and adaptive immunity in a loss of self-tolerance. Deposition and/or in situ production of autoantibodies (e.g. anti-dsDNA) in the glomerulus, activation of complements

(e.g. C3 and C4), T cells and macrophages, production of pro-inflammatory cytokines and chemokines, aberrant micro-RNA expression, cell proliferation and the production of extracellular matrix proteins, are then linked through multiple mechanisms to cause tubular damage, tubulo-interstitial inflammation and fibrosis(7). In addition, genome-wide association studies have highlighted the role of genetic variants predisposing patients to renal damage in SLE(8).

The prevalence and severity of SLE appears to vary among geographical regions and ethnic groups. The variation in prevalence estimates of LN between studies may be due to racial differences in disease prevalence, risk of nephropathy, and varying definitions of criteria for diagnosis of the disease(9–14). The prevalence of LN is higher in blacks (34 – 51%), Hispanics (31 – 43%), and Asians (33 – 55%), than it is in whites (14 – 23%)(9–14). Blacks and Hispanics also tend to present with more severe underlying histopathology, higher serum creatinine concentrations, and more severe proteinuria than whites(15). In addition, blacks, Hispanics and those living in poverty have a worse prognosis than whites and those with a higher socioeconomic status(16). There is growing evidence that in Africa SLE is prevalent and runs a severe course(17). In Cape Town, South Africa, LN is a common cause of chronic kidney disease (CKD) and ESRD requiring dialysis(18). Up to 25% of patients develop ESRD within 10 years of diagnosis of LN, despite recent advances in management(18). Earlier diagnosis, and hence treatment, has a beneficial effect on the prognosis of LN, and it has been shown that late diagnosis is correlated with a higher frequency of renal insufficiency and increased incidence of ESRD(19,20).

The goals of therapy in LN include maintaining the lowest possible disease activity, preventing end-organ damage, minimising drug toxicity, improving quality of life, educating patients (e.g. regarding their role in disease management) and long-term survival. Immunosuppressive agents are important in the treatment of many inflammatory, allergic, immunologic and malignant disorders. Unfortunately, toxicity is one of the commonest causes of iatrogenic illness associated with chronic inflammatory diseases. The side effects profile associated with immunosuppressive therapy is well

documented. These side effects include sepsis, diabetes mellitus, hypothalamic-pituitary-adrenal insufficiency, avascular necrosis of the hip, cushingoid features, and other systemic (e.g. dermatological, musculoskeletal, renal, eye, cardiovascular, gastrointestinal and neuropsychiatric) manifestations(21–24). LN is a serious disease whose prognosis can usually be improved dramatically with immunosuppressive treatment. Current treatment regimens combine corticosteroids with cyclophosphamide, azathioprine, cyclosporin and mycophenolate mofetil. Treatment of LN is prolonged, complex and potentially toxic. In local studies, sepsis was a major complication related to immunosuppressive treatment in LN and often resulted in prolonged hospitalization; sepsis was also a frequent cause of death in these patients (25,26). Determining the appropriate therapeutic regimen for LN requires an accurate assessment of both disease activity and severity, as active disease and quiescent disease can alternate and fluctuate and therefore require treatment modifications(27–31).

Disease activity can be defined as reversible manifestations of an underlying inflammatory process at a point in time in terms of magnitude and intensity(32). Disease severity can be defined as the type and level of organ dysfunction and its consequences(32). The degree of irreversible organ dysfunction is referred to as damage(32). In LN there are generally three patterns of disease activity to consider namely intermittent disease flares, chronically active disease, and quiescent disease. An ongoing challenge in the management of LN is how to continuously and non-invasively evaluate disease activity and hence identify a renal flare. This would allow for the timeous and appropriate commencement of highly potent immunosuppression. At present, LN disease activity and severity are assessed using a combination of clinical symptoms, physical examination and biochemical parameters such as urinalysis, serum creatinine, complement and autoantibodies. However, the correlation between these biomarkers and LN is imperfect, and their utility in reflecting disease activity and in predicting outcome remains controversial(33). They remain unsatisfactory because they lack sensitivity and/or specificity for differentiating renal activity and damage in LN(34). Table 1 demonstrates the diagnostic performance of some of these biomarkers(35).

Table 1: Sensitivity & specificity ranges of biomarkers for active lupus nephritis

TEST	SENSITIVITY	SPECIFICITY
Anti-dsDNA	53 - 100%	50 - 69%
C3	56 - 79%	51 - 64%
C4	53 - 74%	64 - 65%
Anti-C1q	53 - 81%	64 - 71%

** Reproduced from Reyes-Thomas J, Blanco I, Putterman C. Urinary Biomarkers in Lupus Nephritis. 2011*(35)

The histological analysis of kidney tissue is the gold standard for diagnosing and classifying LN and can be used in the assessment of renal disease activity and damage. However, the invasiveness and risk of the procedure, as well as access to this modality, dramatically decrease its pragmatic clinical use(36). Complications include death in 0.02% - 0.1% of patients(36). Serial renal biopsies are therefore not usually performed or recommended. Therefore, novel biomarkers that are able to discriminate disease activity and severity, predict flares, and monitor treatment response and disease progression are clearly required. Non-invasive, easy to obtain, and accurate biomarkers that can be followed-up serially would be of great value in the management of LN patients.

A potential biomarker in LN should be biologically plausible and relevantly involved in the pathogenesis, although it may not be specific for it(37). Candidate biomarkers should optimally

predict the renal histology, while they may also predict long term outcomes such as deterioration in renal function or renal death requiring dialysis or transplantation. Biomarkers should perform better than the standard-of-care tests, but also show significant correlation with them(38). They should be cost-effective, reasonably simple to measure routinely and easily interpretable. In addition, they should be sensitive to change and thus appropriate for the serial monitoring of disease activity, treatment response, remission and relapse(37). Furthermore, they should optimally have the ability to predict changes before alterations in standard clinical parameters become apparent so that early treatment, or preventative strategies, can be implemented promptly(34). Finding biomarkers that fulfill these criteria will help in disease activity evaluation, identify patients at risk for kidney damage, and facilitate early diagnosis and intervention to improve favourable outcomes.

Urinary biomarkers are easily obtained by non-invasive means and reflect current renal status, as they specifically represent local inflammatory activity(39). In health, 70% of the protein content within the urine originates in the urinary tract, whereas only 30% originates from filtered plasma(40). Instrumental in the pathogenesis of LN, cytokines and chemokines (secreted locally in the kidneys) are expelled in urine samples(39,41). Furthermore, it has been shown that the excretion of these biomarkers in the urine is a good indicator of their local production and secretion(35,42,43). Urinary biomarkers such as monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-like weak inducer of apoptosis (TWEAK) may therefore more accurately reflect renal inflammatory disease activity in LN than serum markers.

1.2.2 Monocyte chemoattractant protein-1 (MCP-1)

Monocyte chemoattractant protein-1 is a low molecular weight member of the CC chemokine subfamily(44). Chemokines include proteins that play central roles in many biological and pathological processes. Chemokines were initially identified as regulators of leukocyte trafficking, with subsequent studies demonstrating their involvement in other aspects of the inflammatory process, such as tissue

remodeling, angiogenesis and fibrosis(45–48). Chemokines have multiple actions and functions, and thus play a major role in various auto-immune diseases, allergic disorders and transplant rejections, and in vascular, neoplastic and infectious conditions. Changes in chemokine expression or function lead to the persistence of an inflammatory reaction beyond its original purpose thereby creating a key pathogenic event for the establishment of chronic inflammation(49).

The role of MCP-1 as a potent monocyte attractant linked to innate immunity is well documented(50). MCP-1 exerts its effects through binding to G-protein-coupled receptors, namely CCR2, on the surface of leukocytes targeted for activation and migration(49). MCP-1 attracts T cells, natural killer cells, and basophils in acute inflammatory conditions, and acts as an important mediator in chronic inflammation(44).

SLE patients have higher serum MCP-1 levels than healthy controls, even in the absence of symptoms(51). MCP-1 has been shown to play a major role in inflammatory processes in renal disease linked to SLE and have been reported to increase with the progression of disease activity in patients with LN(52–57). In animal models, MCP-1 antagonism or gene therapy ameliorated these inflammatory effects, and MCP-1 knockout mice were less prone to LN(58–60). Being a small protein, MCP-1 is easily filtered by the glomerulus from the plasma into the urine. MCP-1 is also produced locally in the kidneys by renal mesangial cells, endothelial cells, tubular epithelial cells, and smooth muscle cells(49). In humans, when compared to a wide range of cytokines, urinary MCP-1 (uMCP-1) performed better as a marker of disease activity in SLE, was reported to correlate with LN disease activity, and seemed to have the potential to predict renal flares(38,43,49,61). In a 2012 study by Barbado et al, uMCP-1, when compared with 26 other cytokines, chemokines and cellular growth factors, was shown to be the best biomarker for detection of disease activity in SLE(49). In addition, uMCP-1 levels have been shown to be significantly correlated with both LN class and severity of LN flare, and to decline in response to treatment(38,62). Thus uMCP-1 is a good candidate as a non-invasive biomarker for assessing LN disease activity.

1.2.3 Tumour necrosis factor-like weak inducer of apoptosis (TWEAK)

The cytokine TWEAK was first discovered in 1997 and assigned to the tumour necrosis factor (TNF) superfamily, the members of which play pivotal roles in regulating the immune system(63). TWEAK was named for its relation to TNF, proinflammatory effects and weak ability to promote cell death. The human TWEAK gene encodes a 249 amino acid type II transmembrane glycoprotein which is proteolytically cleaved into a soluble form that circulates as a trimer believed to be the primary mediator of its effects(64). The major source of circulating TWEAK is activated monocytes and macrophages although it is expressed in many tissues. In the kidneys, both resident kidney cells and infiltrating leucocytes express TWEAK(65). Currently the only known TWEAK receptor is fibroblast growth factor inducible 14 (Fn14)(64).

Evidence has developed supporting a role for TWEAK activation of intrinsic renal cell Fn14 receptors in the pathogenesis of various kidney diseases, including LN(65). In renal cells TWEAK mediates the following effects: proliferation, differentiation, migration, enhancement of cell survival, tissue regeneration, modulation of cell death and apoptosis, upregulation of proinflammatory mediators (multiple chemokines, cytokines and adhesion molecules) and neoangiogenesis(63–68). Human mesangial cells, podocytes and tubular cells have all been found to express Fn14 and renal biopsies of LN patients have demonstrated strong glomerular and tubulointerstitial staining for Fn14(69). TWEAK induces the expression of multiple inflammatory mediators, including RANTES, MCP-1, IP-10, MIP-1á, ICAM-1, VCAM-1, MMP-1, and MMP-9(68). In addition, recent studies have shown that TWEAK significantly stimulated proliferation of mesangial cells and podocytes(64).

Based on these considerations urinary TWEAK (uTWEAK) may be a useful biomarker in patients with LN. TWEAK may be even more revealing than individual chemokines, since TWEAK is proximal in the inflammatory cascade and induces several nephritis-related mediators(35). TWEAK effects downstream expression of inflammatory messengers, including MCP-1, in human mesangial cells,

podocytes, and tubular cells(68,70). In early multicentre studies of SLE patients by Schwartz and colleagues, uTWEAK was found to be higher in those with LN, than in control groups (overall significance p=0.039; SLE non-LN p=0.005; healthy control p=0.003; rheumatoid arthritis p=0.013). Additionally, they found uTWEAK levels would peak during a LN flare, and decrease after the flare, indicating its potential usefulness as a marker of LN disease activity(39,62). More recently, studies in Egypt(71–74), China(75,76), Colombia(77), Korea(78), Mexico(79) and Thailand(80) have confirmed uTWEAK's potential as a biomarker in diverse populations of LN.

1.2.4 Hypothesis

Urinary levels of MCP-1 and TWEAK will reflect the level of renal disease activity in patients with LN. The levels of these biomarkers will be significantly increased in patients with active LN when compared with patients with quiescent LN. The levels of these biomarkers will decrease with response to treatment. The levels of these biomarkers will correlate with other standard tests of disease activity status in LN.

1.2.5 Rationale for the Research

Lupus nephritis is regarded as the most serious common complication of SLE. The relapsing and remitting course of LN requires close surveillance of disease activity status and appropriate treatment modifications throughout patients' lives. Judicious use of immunosuppression in patients with active LN is required as the treatment may have serious complications. Although renal biopsy remains the gold standard to assess disease activity status, repeat biopsies are not recommended, rendering the procedure impractical as a clinical monitoring tool. Current modalities for assessing disease activity status include clinical scores and laboratory tests such as urinalysis, serum creatinine, complement and autoantibodies. The diagnostic performance of these modalities remains unsatisfactory. A non-

invasive and reliable biomarker that reflects LN disease activity status is therefore highly desirable. Preliminary studies have shown that uMCP-1 and uTWEAK correlate with disease activity, and may predict renal flares, response to treatment and histology. These biomarkers may also enhance patient management, thereby decreasing morbidity and mortality.

Few studies have assessed uMCP-1 and uTWEAK in the same cohort of patients, and to the best of our knowledge no studies have been performed in a South African cohort of patients. Previous studies were limited in length of follow-up to less than six-months. Furthermore, previous studies reported either absolute concentrations of the biomarkers or concentrations corrected for urinary creatinine, not both. Moreover, some studies did not demonstrate disease activity in the active group with the gold-standard renal biopsy.

In this study, uMCP-1 and uTWEAK were assessed in a cohort of South African patients with active and quiescent LN at baseline, and longitudinally at six-months and one-year. We reported both absolute concentrations of the biomarkers and values corrected for urinary creatinine, and compared their usefulness. Lastly, the active group in our study had a recent renal biopsy demonstrating disease activity.

1.2.6 Research Setting

For this study the cohort of patients were recruited from the Department of Medicine, Division of Nephrology and Hypertension, Groote Schuur Hospital (GSH), Cape Town, South Africa, over a two-year period. GSH is a large, government-funded, teaching hospital. The hospital serves the central health district of the Cape Town Metro region, with a diverse socioeconomic and cultural population.

In this prospective observational study, the patients were each followed up over a one-year period. The patients were newly diagnosed with LN (biopsy proven) or known LN patients with a previous renal biopsy. Two groups of patients were identified depending on their current disease activity status:

1. Active disease group
2. Quiescent disease group

1.3 Ethical Considerations

Ethical approval for this study was granted by the University of Cape Town Human Research Ethics Committee (HREC), as a sub-study of the ongoing ALUGEN Registry(81) of patients with SLE in Cape Town. Please see Appendix 1 for the relevant documentation pertaining to the study's formal approval (HREC/REF:402/2014 and HREC/REF:856/2016)) and the annual progress report and renewal forms (FHS016).

Signed informed consent was obtained from all patients enrolling in the study. Please see Appendix 2 for a copy of the patient consent form. Strict adherence to patient confidentiality and anonymity was maintained. Patient information was recorded in a database for sole use by the researchers involved in the study. See Appendix 3 for the ALUGEN Registry summary and Appendix 4 for the Data Sheets. Patients names were encoded and did not appear on the sample tubes used during the study. Appendices 5 and 6 detail the scoring systems using SLEDAI-2K and SLICC, respectively.

Important ethical issues that were considered in the development of this study included the collection of patient samples (blood and urine), access to participant information and medical records, and the confidentiality thereof.

1.4 Author Guidelines for LUPUS

Author guidelines for article submission to *Lupus* (in their exact words) are attached in Appendix 7.

1.5 References

1. Cook HT, Botto M. Mechanisms of disease: The complement system and the pathogenesis of systemic lupus erythematosus. Nature Clinical Practice Rheumatology. 2006.
2. Hrycek E, Franek A, Błaszczak E, Dworak J, Hrycek A. Serum levels of selected chemokines in systemic lupus erythematosus patients. Rheumatol Int. 2013;33(9):2423–7.
3. Davis LS, Hutcheson J, Mohan C. The role of cytokines in the pathogenesis and treatment of systemic lupus erythematosus. J Interf Cytokine Res. 2011;
4. Cameron JS. Lupus nephritis. J Am Soc Nephrol. 1999;10(2):413–24.
5. Michaelson JS, Wisniacki N, Burkly LC, Putterman C. Role of TWEAK in lupus nephritis: A bench-to-bedside review. Journal of Autoimmunity. 2012.
6. Hopkinson ND, Doherty M, Powell RJ. Clinical features and race-specific incidence/prevalence rates of systemic lupus erythematosus in a geographically complete cohort of patients. Ann Rheum Dis. 1994;
7. Lech M, Anders HJ. The pathogenesis of lupus nephritis. Journal of the American Society of Nephrology. 2013.
8. Chung S a, Brown EE, Williams AH, Ramos PS, Berthier CC, Bhangale T, et al. Lupus Nephritis Susceptibility Loci in Women with Systemic Lupus Erythematosus. J Am Soc Nephrol. 2014;
9. Wang F, Wang CL, Tan CT, Manivasagar M. Systemic lupus erythematosus in Malaysia: A study of 539 patients and comparison of prevalence and disease expression in different racial and gender groups. Lupus. 1997;
10. Alarcón GS, McGwin G, Petri M, Reveille JD, Ramsey-Goldman R, Kimberly RP, et al. Baseline characteristics of a multiethnic lupus cohort: PROFILE. Lupus. 2002;
11. Bastian HM, Roseman JM, McGwin G, Alarcón GS, Fessler BJ, Reveille JD. Systemic lupus erythematosus in three ethnic groups. XII. Risk factors for lupus nephritis after diagnosis. Lupus. 2002;
12. Feldman CH, Hiraki LT, Liu J, Fischer MA, Solomon DH, Alarcõn GS, et al. Epidemiology and sociodemographics of systemic lupus erythematosus and lupus nephritis among US adults with Medicaid coverage, 2000-2004. Arthritis Rheum. 2013;
13. Hiraki LT, Feldman CH, Liu J, Alarcõn GS, Fischer MA, Winkelmayer WC, et al. Prevalence, incidence, and demographics of systemic lupus erythematosus and lupus nephritis from 2000 to 2004 among children in the US medicaid beneficiary population. Arthritis Rheum. 2012;
14. Seligman VA, Lum RF, Olson JL, Li H, Criswell LA. Demographic differences in the

development of lupus nephritis: A retrospective analysis. Am J Med. 2002;

15. Contreras G, Lenz O, Pardo V, Borja E, Cely C, Iqbal K, et al. Outcomes in African Americans and Hispanics with lupus nephritis. Kidney Int. 2006;

16. Korbet SM, Schwartz MM, Evans J, Lewis EJ. Severe lupus nephritis: Racial differences in presentation and outcome. J Am Soc Nephrol. 2007;

17. Tiffin N, Hodkinson B, Okpechi I. Lupus in Africa: Can we dispel the myths and face the challenges? Lupus. 2014;23(1):102–11.

18. Okpechi I, Swanepoel C, Duffield M, Mahala B, Wearne N, Alagbe S, et al. Patterns of renal disease in Cape Town South Africa : a 10-year review of a single-centre renal biopsy database. Nephrol Dial Transpl. 2011;(26):1853–61.

19. Esdaile JM, Joseph L, Mackenzie T, Kashgarian M, Hayslett JP. The benefit of early treatment with immunosuppressive agents in lupus nephritis. J Rheumatol. 1994;

20. Faurschou M, Starklint H, Halberg P, Jacobsen S. Prognostic factors in lupus nephritis: Diagnostic and therapeutic delay increases the risk of terminal renal failure. J Rheumatol. 2006;

21. Hoes JN, Jacobs JWG, Verstappen SMM, Bijlsma JWJ, Van Der Heijden GJMG. Adverse events of low- to medium-dose oral glucocorticoids in inflammatory diseases: A meta-analysis. Ann Rheum Dis. 2009;

22. Curtis JR, Westfall AO, Allison J, Bijlsma JW, Freeman A, George V, et al. Population-based assessment of adverse events associated with long-term glucocorticoid use. Arthritis Care and Research. 2006.

23. Fardet L, Flahault A, Kettaneh A, Tiev KP, Généreau T, Tolédano C, et al. Corticosteroid-induced clinical adverse events: Frequency, risk factors and patient's opinion. Br J Dermatol. 2007;

24. Fries JF, Williams CA, Ramey D, Bloch DA. The relative toxicity of disease-modifying antirheumatic drugs. Arthritis Rheum. 1993;

25. Ayodele OE, Okpechi IG, Swanepoel CR. Long-term renal outcome and complications in South Africans with proliferative lupus nephritis. Int Urol Nephrol. 2013;

26. Okpechi IG, Ayodele OE, Jones ESW, Duffield M, Swanepoel CR. Outcome of patients with membranous lupus nephritis in Cape Town South Africa. Nephrol Dial Transplant. 2012;

27. Liang MH, Socher SA, Larson MG, Schur PH. Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. Arthritis Rheum. 1989;

28. Vitali C, Bencivelli W, Isenberg DA, Smolen JS, Snaith ML, Sciuto M, et al. Disease activity in systemic lupus erythematosus: Report of the Consensus Study Group of the European Workshop for Rheumatology Research. I. A descriptive analysis of 704 European lupus patients. In: Clinical and Experimental Rheumatology. 1992.

29. Vitali C, Bencivelli W, Isenberg DA, Smolen JS, Snaith ML, Sciuto M, et al. Disease

activity in systemic lupus erythematosus: Report of the Consensus Study Group of the European Workshop for Rheumatology Research. II. Identification of the variables indicative of disease activity and their use in the development of an acticity sc. In: Clinical and Experimental Rheumatology. 1992.

30. Bencivelli W, Vitali C, Isenberg DA, Smolen JS, Snaith ML, Sciuto M, et al. Disease activity in systemic lupus erythematosus: Report of the Consensus Study Group of the European Workshop for Rheumatology Research. III. Development of a computerised clinical chart and its application to the comparison of different indices of disea. In: Clinical and Experimental Rheumatology. 1992.

31. Gladman DD, Ibañez D, Urowltz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol. 2002;

32. Gladman DD, Goldsmith CH, Urowitz MB, Bacon P, Fortin P, Ginzler E, et al. The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index for systemic lupus erythematosus international comparison. J Rheumatol. 2000;

33. Illei GG, Tackey E, Lapteva L, Lipsky PE. Biomarkers in systemic lupus erythematosus II. Markers of disease activity. Arthritis and Rheumatism. 2004.

34. Mok CC. Biomarkers for Lupus Nephritis: A Critical Appraisal. J Biomed Biotechnol. 2010;

35. Reyes-Thomas J, Blanco I, Putterman C. Urinary biomarkers in lupus nephritis. Clin Rev Allergy Immunol. 2011;40(3):138–50.

36. Whittier WL. Complications of the Percutaneous Kidney Biopsy. Adv Chronic Kidney Dis. 2012;19(3):179–87.

37. Liu CC, Kao AH, Manzi S, Ahearn JM. Biomarkers in systemic lupus erythematosus: Challenges and prospects for the future. Ther Adv Musculoskelet Dis. 2013;

38. Li Y, Tucci M, Narain S, Barnes E V., Sobel ES, Segal MS, et al. Urinary biomarkers in lupus nephritis. Autoimmun Rev [Internet]. 2006 Jul;5(6):383–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S156899720500203X

39. Schwartz N, Michaelson JS, Putterman C. Lipocalin-2, TWEAK, and other cytokines as urinary biomarkers for lupus nephritis. Ann N Y Acad Sci. 2007;1109:265–74.

40. Peredo G De, Breuil B. Urine in Clinical Proteomics *. Mol Cell Proteomics. 2008;1850–62.

41. Kelley VR, Rovin BH. Chemokines: Therapeutic targets for autoimmune and inflammatory renal disease. Springer Seminars in Immunopathology. 2003.

42. Rovin B, Kollaros M, Schwartz N, Michaelson JS, Burkly LC, Aranow C, et al. Urinary TWEAK and the activity of lupus nephritis. J Autoimmun. 2007;27(4):242–50.

43. Wada T, Yokoyama H, Su SB, Mukaida N, Iwano M, Dohi K, et al. Monitoring urinary levels of monocyte chemotactic and activating factor reflects disease activity of lupus nephritis. Vol. 49, Kidney International. 1996. p. 761–7.

44. Yadav A, Saini V, Arora S. MCP-1: Chemoattractant with a role beyond immunity: A review. Clin Chim Acta [Internet]. 2010;411(21–22):1570–9. Available from: http://dx.doi.org/10.1016/j.cca.2010.07.006

45. Schall TJ, Bacon KB. Chemokines, leukocyte trafficking, and inflammation. Curr Opin Immunol. 1994;

46. Lukacs NW. Role of chemokines in the pathogenesis of asthma. Nature Reviews Immunology. 2001.

47. Matsukawa A, Hogaboam CM, Lukacs NW, Kunkel SL. Chemokines and innate immunity. Reviews in Immunogenetics. 2000.

48. Lukacs-Kornek V, Engel D, Tacke F, Kurts C. The role of chemokines and their receptors in dendritic cell biology. Frontiers in Bioscience. 2008.

49. Barbado J, Martin D, Vega L, Almansa R, Gonçalves L, Nocito M, et al. MCP-1 in urine as biomarker of disease activity in Systemic Lupus Erythematosus. Cytokine [Internet]. 2012;60(2):583–6. Available from: http://dx.doi.org/10.1016/j.cyto.2012.07.009

50. Melgarejo E, Medina MÁ, Sánchez-Jiménez F, Urdiales JL. Monocyte chemoattractant protein-1: A key mediator in inflammatory processes. International Journal of Biochemistry and Cell Biology. 2009.

51. Vega L, Barbado J, Almansa R, González-Gallego R, Rico L, Jimeno A, et al. Prolonged standard treatment for systemic lupus erythematosus fails to normalize the secretion of innate immunity-related chemokines. Eur Cytokine Netw. 2010;

52. Barbado J, Vega L, González-Gallego R, Jimeno A, De Lejarazu RO, Bermejo-Martin JF. MCP-1 in urine as biomarker of renal lupus in absence of cytokines, interferon-γ and growth factors. Reumatol Clínica (English Ed. 2010;

53. Alzawawy A, Zohary M, Ablordiny M, Eldalie M. Estimation of monocyte-chemoattractantprotein-1 (Mcp-1) level in patients with lupus nephritis. Int J Rheum Dis. 2009;12(4):311–8.

54. Kiani AN, Johnson K, Chen C, Diehl E, Hu H, Vasudevan G, et al. Urine osteoprotegerin and monocyte chemoattractant protein-1 in lupus nephritis. J Rheumatol. 2009;

55. Marks SD, Williams SJ, Tullus K, Sebire NJ. Glomerular expression of monocyte chemoattractant protein-1 is predictive of poor renal prognosis in paediatric lupus nephritis. Nephrol Dial Transplant. 2008;

56. Wągrowska-Danilewicz M, Stasikowska O, Danilewicz M. Correlative insights into immunoexpression of monocyte chemoattractant protein-1, transforming growth factor β-1 and CD68+ cells in lupus nephritis. Polish J Pathol. 2005;

57. Kaneko H, Ogasawara H, Naito T, Akimoto H, Lee S, Hishikawa T, et al. Circulating levels of β-chemokines in systemic lupus erythematosus. J Rheumatol. 1999;

58. Hasegawa H, Kohno M, Sasaki M, Inoue A, Ito MR, Terada M, et al. Antagonist of monocyte chemoattractant protein 1 ameliorates the initiation and progression of lupus nephritis and renal vasculitis in MRL/lpr mice. Arthritis Rheum. 2003;

59. Shimizu S, Nakashima H, Masutani K, Inoue Y, Miyake K, Akahoshi M, et al. Anti-monocyte chemoattractant protein-1 gene therapy attenuates nephritis in MRL/lpr mice. Rheumatology. 2004;

60. Tesch GH, Maifert S, Schwarting A, Rollins BJ, Kelley VR. Monocyte Chemoattractant Protein 1–Dependent Leukocytic Infiltrates Are Responsible for Autoimmune Disease in Mrl- Fas lpr Mice . J Exp Med. 1999;

61. Rovin BH, Zhang X. Biomarkers for lupus nephritis: The quest continues. Clinical Journal of the American Society of Nephrology. 2009.

62. Schwartz N, Rubinstein T, Burkly LC, Collins CE, Blanco I, Su L, et al. Urinary TWEAK as a biomarker of lupus nephritis: A multicenter cohort study. Arthritis Res Ther. 2009;11(5):1–10.

63. Chicheportiche Y, Bourdon PR, Xu H, Hsu YM, Scott H, Hession C, et al. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J Biol Chem. 1997;

64. Putterman C, Mathieson PW, Berman JW, Saleem MA, Jarchum I, Gao H-X, et al. TNF-like weak inducer of apoptosis (TWEAK) induces inflammatory and proliferative effects in human kidney cells. Cytokine [Internet]. 2009;46(1):24–35. Available from: http://dx.doi.org/10.1016/j.cyto.2008.12.001

65. Egido J, Ortiz A, Ucero AC, Ramos AM, Sanchez-Nino MD, Ruiz-Ortega M, et al. TWEAK and the progression of renal disease: clinical translation. Nephrol Dial Transplant. 2014;29(suppl 1):i54–62.

66. Lynch CN, Wang YC, Lund JK, Chen YW, Leal JA, Wiley SR. TWEAK induces angiogenesis and proliferation of endothelial cells. J Biol Chem. 1999;

67. Zhao Z, Burkly LC, Campbell S, Schwartz N, Molano A, Choudhury A, et al. TWEAK/Fn14 Interactions Are Instrumental in the Pathogenesis of Nephritis in the Chronic Graft-versus-Host Model of Systemic Lupus erythematosus. J Immunol. 2007;

68. Browning B, Schiffer L, Campbell S, Putterman C, Zheng T, Berman JW, et al. Proinflammatory Effects of Tweak/Fn14 Interactions in Glomerular Mesangial Cells. J Immunol. 2014;176(3):1889–98.

69. Bekhit M, Kamel N, Mohammed M, Abaza N, Saad El Din S. Role of tumor necrosis factor-like weak inducer of apoptosis/fibroblast growth factor-inducible molecule 14 pathway in lupus nephritis. Egypt Rheumatol Rehabil [Internet]. 2018;45(4):182. Available from: http://www.err.eg.net/downloadpdf.asp?issn=1110-161X;year=2017;volume=44;issue=4;spage=153;epage=158;aulast=Saba;type=2

70. Chen J, Wei L, Xia Y. Roles of tumour necrosis factor-related weak inducer of apoptosis/fibroblast growth factor-inducible 14 pathway in lupus nephritis. Nephrology. 2017.

71. El-Shehaby A, Darweesh H, El-Khatib M, Momtaz M, Marzouk S, El-Shaarawy N, et al. Correlations of urinary biomarkers, TNF-like weak inducer of apoptosis (TWEAK), osteoprotegerin (OPG), monocyte chemoattractant protein-1 (MCP-1), and IL-8 with lupus nephritis. J Clin Immunol. 2011;31(5):848–56.

72. Abd El-Fattah El-Feqi M, Mohamed RA, Salem MN, Taha HA, Eesa NN. Urinary TNF-like weak inducer of apoptosis (TWEAK) as a biomarker of lupus nephritisTNF-ähnlicher schwacher Induktor von Apoptose (TWEAK) im Urin als Biomarker einer Lupusnephritis. Z Rheumatol. 2016;77(1):71–7.

73. Elsayed M, Elhefnawy KA, Qassem AA, Amr GE. Urinary Tumor Necrosis Factor-Like Weak Inducer of Apoptosis (TWEAK) As a Marker for Lupus Nephritis Activity. Int J Sci Res. 2013;4(2):1615–9.

74. Selim ZI, Khader TM, Mohammed HO, Al-Hammady DH, Seif HMA, Al-Johi AA. Urinary Tumor Necrosis Factor-Like Weak Inducer of Apoptosis (uTWEAK) as a biomarker for lupus nephritis activity and its correlation with histolopathological findings of renal biopsy. Egypt Rheumatol [Internet]. 2018; Available from: https://doi.org/10.1016/j.ejr.2018.01.008

75. Xuejing Z, Jiazhen T, Jun L, Xiangqing X, Shuguang Y, Fuyou L. Urinary TWEAK level as a marker of lupus nephritis activity in 46 cases. J Biomed Biotechnol. 2012;2012(March 2009):1–7.

76. Zhu P, Li ZQ, Zheng ZH, Dong XW, Ding J, Shi JH, et al. Combined detection of uMCP-1 and uTWEAK for rapid discrimination of severe lupus nephritis. Lupus. 2018;096120331875850.

77. Gómez-Puerta JA, Ortiz Reyes BL, Urrego T, Vanegas AL, Muñoz CH, Restrepo M, et al. A panel of urinary biomarkers to assess renal involvement in latin american patients with systemic lupus erythematosus. Arthritis Rheumatol [Internet]. 2016;68:158–60. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L613887196%5Cnhttp://dx.doi.org/10.1002/art.39977%5Cnhttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=23265205&id=doi:10.1002%2Fart.39977&atitle=A+panel+of+urinary+biomarkers+to+asse

78. Choe JY, Kim SK. Serum TWEAK as a biomarker for disease activity of systemic lupus erythematosus. Inflamm Res. 2016;65(6):479–88.

79. Reyes-Martínez F, Pérez-Navarro M, Rodríguez-Matías A, Soto-Abraham V, Gutierrez-Reyes G, Medina-Avila Z, et al. Assessment of urinary TWEAK levels in Mexican patients with untreated lupus nephritis: An exploratory study. Nefrologia [Internet]. 2018;38(2):152–60. Available from: http://dx.doi.org/10.1016/j.nefro.2017.04.005

80. Suttichet TB, Kittanamongkolchai W, Phromjeen C, Anutrakulchai S, Panaput T, Ingsathit A, et al. Urine TWEAK level as a biomarker for early response to treatment in active lupus nephritis: a prospective multicentre study. Lupus Sci Med [Internet]. 2019;6(1):e000298. Available from: http://lupus.bmj.com/lookup/doi/10.1136/lupus-2018-000298

81. Mapiye D, Hodkinson B, Okpechi I, Jayne D, Tiffin N, Kalla A. The African Lupus Genetics Network (ALUGEN) registry: standardized, prospective follow-up studies in African patients with systemic lupus erythematosus. Lupus. 2015;25(3):325–30.

82. Wu H, Zeng J, Yin J, Peng Q, Zhao M, Lu Q. Organ-specific biomarkers in lupus. Autoimmun Rev. 2017;16(4):391–7.

83. Li Y, Fang X, Li QZ. Biomarker Profiling for Lupus Nephritis. Genomics, Proteomics Bioinforma [Internet]. 2013;11(3):158–65. Available from: http://dx.doi.org/10.1016/j.gpb.2013.05.003

84. Rodríguez-Castro S, Rosetti F, Flores-Mendoza G, Sansón SP, Crispín JC. Mechanisms of Tissue Injury in Lupus Nephritis. Trends Mol Med. 2018;24(4):364–78.

85. Yadav A, Saini V, Arora S. MCP-1: Chemoattractant with a role beyond immunity: A review. Clinica Chimica Acta. 2010.

86. Xia Y, Herlitz LC, Gindea S, Wen J, Pawar RD, Misharin A, et al. Deficiency of Fibroblast Growth Factor-Inducible 14 (Fn14) Preserves the Filtration Barrier and Ameliorates Lupus Nephritis. J Am Soc Nephrol. 2014;

87. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis and rheumatism. 1997.

88. Gladman DD, Urowitz MB. The SLICC/ACR damage index: Progress report and experience in the field. Lupus. 1999;8(8):632–7.

89. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro III AF, Feldman HI, et al. A New Equation to Estimate Glomerular Filtration Rate Disclosure of conflicts of interest: We have received confirmation from Drs. Ann Intern Med. 2009;150(9):604–12.

90. Hill GS, Delahousse M, Nochy D, Tomkiewicz E, Remy P, Mignon F, et al. A new morphologic index for the evaluation of renal biopsies in lupus nephritis. Kidney Int. 2000;

91. Kidney Disease Improving Global Outcomes. KDIGO Clinical practice guideline for glomerulonephritis. Kidney Int Suppl Int Suppl. 2012;

92. El Hamshary A, Abd El Raheim Y, Mohamed A, Talaat A, Ghobrial E. Urinary monocyte chemoattractant protein-1 as a biomarker of lupus nephritis activity in children. Saudi J Kidney Dis Transplant. 2015;26(3):507.

93. Lee YH, Song GG. Urinary MCP-1 as a biomarker for lupus nephritis: a meta-analysis. Z Rheumatol [Internet]. 2017;76(4):357–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27278779

94. Lee YH, Song GG. Urinary Tumor Necrosis Factor-like Weak Inducer of Apoptosis as a Biomarker for Lupus Nephritis: A Meta-analysis. J Rheum Dis [Internet]. 2017;24(2):85. Available from: https://synapse.koreamed.org/DOIx.php?id=10.4078/jrd.2017.24.2.85

95. Shah SA, Abdul Gafor AH, Kong NCT, Mohd M, Ba'in A, Alharazy S. Urine Monocyte Chemoattractant Protein-1 and Lupus Nephritis Disease Activity: Preliminary Report of a Prospective Longitudinal Study. Autoimmune Dis. 2015;2015:1–13.

96. Reyes-Martínez F, Pérez-Navarro M, Rodríguez-Matías A, Soto-Abraham V, Gutierrez-Reyes G, Medina-Avila Z, et al. Assessment of urinary TWEAK levels in Mexican patients with untreated lupus nephritis: An exploratory study. Nefrologia [Internet]. 2018;38(2):152–60. Available from: https://doi.org/10.1016/j.nefroe.2018.02.006

97. Waikar SS, Sabbisetti VS, Bonventre J V. Normalization of urinary biomarkers to

creatinine during changes in glomerular filtration rate. Kidney Int [Internet]. 2010;78(5):486–94. Available from: http://dx.doi.org/10.1038/ki.2010.165

98. Tang KWA, Toh QC, Teo BW. Normalisation of urinary biomarkers to creatinine for clinical practice and research – When and why. Singapore Med J. 2015;56(1):7–10.

99. Reid CN, Stevenson M, Abogunrin F, Ruddock MW, Emmert-Streib F, Lamont J V., et al. Standardization of Diagnostic Biomarker Concentrations in Urine: The Hematuria Caveat. PLoS One. 2012;7(12).

100. Lewis EC, Taylor TP, Oates JC, Janech MG, Arthur JM, Slate EH. Overcoming the Effects of Matrix Interference in the Measurement of Urine Protein Analytes. Biomark Insights. 2012;7:BMI.S8703.

CHAPTER TWO: PUBLICATION-READY MANUSCRIPT

Title

Using urinary MCP-1 and TWEAK to assess disease activity in a cohort of South African patients with lupus nephritis

Authorship

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Keywords

MCP-1, monocyte chemoattractant protein-1; TWEAK, tumour necrosis factor-like weak inducer of apoptosis; urinary biomarkers; lupus nephritis; systemic lupus erythematosus; disease activity; renal biopsy

Abstract

Background: Renal involvement is common in systemic lupus erythematosus (SLE) and can lead to chronic kidney disease. Diagnosis of lupus nephritis (LN) is dependent on renal biopsy. Due to its invasiveness, repeat renal biopsy for monitoring disease activity is not recommended, thus creating a need for non-invasive and accurate biomarkers. Monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-like weak inducer of apoptosis (TWEAK) have been implicated in the pathogenesis of LN and are thus potential biomarkers for disease activity monitoring.

Methods: In this study urinary MCP-1 (uMCP-1) and TWEAK (uTWEAK), together with standard markers of disease activity, were analysed in a cohort of 50 biopsy-proven LN patients at baseline, after six-months of induction therapy, and at one-year.

Results: At baseline, there was correlation between uMCP-1 and uTWEAK. Both biomarkers also correlated with standard of care tests, and clinical scores. The median [interquartile range] of uMCP-1 and uTWEAK were significantly increased in the active group when compared to the quiescent group (1440 [683–2729] vs 256 [175–477] pg/mL, $p<0.0001$, and 209 [117–312] vs 74 [11–173] pg/mL, $p=0.0008$, respectively). After completion of induction therapy in the active group, there was no significant difference in biomarker results between the groups. The sensitivity and specificity for indicating disease activity was 95% and 73% for uMCP-1, and 60% and 90% for uTWEAK, respectively,.

Conclusions: uMCP-1 and uTWEAK reflect LN disease activity, and correlate with standard of care biomarkers in a South African cohort, however, further studies are needed to assess additional clinical benefit.

Introduction

Background and Rationale

An unmet need in the management of lupus nephritis (LN) is how to regularly and non-invasively evaluate disease activity and severity, as this would allow for the judicious utilisation of appropriate therapeutic regimens. Although renal biopsy remains the gold standard, invasiveness, risk and access to the procedure dramatically decrease its pragmatic clinical use. At present, disease activity and severity are assessed using a combination of clinical and biochemical parameters. Standard of care biomarkers include proteinuria and urinalysis, serum creatinine, complement (C3 and C4) and various autoantibodies. However, the correlation between these biomarkers and LN is imperfect, restricted by low sensitivities or specificities(1). Their utility in accurately reflecting renal disease activity and in predicting outcomes remains controversial(2).

Within the kidney, locally secreted chemokines such as monocyte chemoattractant protein-1 (MCP-1) and cytokines such as tumour necrosis factor-like weak inducer of apoptosis (TWEAK) are instrumental in the pathogenesis of LN(3–5). The role of MCP-1 and its receptor chemokine receptor 2 (CCR2) as a potent monocyte attractant linked to innate immunity is well documented(6). Briefly, MCP-1 attracts T cells, natural killer cells, and basophils in acute inflammatory conditions, and acts as an important mediator in chronic inflammation(7). Furthermore, MCP-1 is involved in other aspects of the inflammatory process, such as fibrosis, tissue remodelling and angiogenesis(7).

The cytokine TWEAK is expressed by both infiltrating leucocytes (monocytes and macrophages) and resident kidney cells(1). Initially membrane-bound, TWEAK is proteolytically processed into a soluble form that circulates as a trimer believed to be the

primary mediator of its biological effects via its receptor fibroblast growth factor-inducible 14 (Fn14) which is expressed by various cells in the kidney (8,9). Proximal in this inflammatory pathway, TWEAK/Fn14 stimulates mesangial cells to secrete pro-inflammatory cytokines like MCP-1, RANTES, IP-10, CXCL-1 and CVCAM-1, which lead to the recruitment of activated T cells and mediates other biological processes such as cell growth, tissue remodelling, angiogenesis, fibrosis and apoptosis(8).

Excretion of MCP-1 and TWEAK in the urine has been shown to be a good indicator of their local production and secretion(1,4). Therefore, these two novel urinary biomarkers may accurately reflect renal inflammatory disease activity in LN. Few studies have assessed them in combination and, to the best of our knowledge, no studies have been performed in a South African cohort of patients. In this study, the urinary levels of MCP-1 (uMCP-1) and TWEAK (uTWEAK) were assessed in patients with active and quiescent LN at baseline and longitudinally at six-months and one-year.

Materials and Methods

Study Design and Population

In this prospective observational study, a cohort of fifty patients with biopsy-proven LN was recruited from the Nephrology Clinic at Groote Schuur Hospital in Cape Town during the period August 2016 to December 2017. This consisted of a clinically stable group with quiescent LN (sample number [n] =30) and a group with clinically active LN (n=20). The quiescent group were patients known to have LN, confirmed in the past with renal biopsy, and followed up at the nephrology clinic. Inactive disease status was confirmed clinically by

history, physical examination, disease activity scores, and biochemical assessments (urine and serum). The active group were newly diagnosed patients with LN (confirmed on renal biopsy) or known patients with current disease relapse with repeat biopsy confirming active disease. Participants who consented to take part in this study were 18 years or older, and diagnosed with SLE according to ACR criteria(10). Patients not included were those with co-morbid HIV infection, SLE patients with renal disease but with no renal biopsy, those with inadequate renal biopsy, or if the biopsy indicated alternative or dual renal pathologies. Approval for this study was obtained from the University of Cape Town Human Research Ethics Committee.

Study Data Collection

At baseline, relevant demographic, clinical and biochemical data were collected. SLE Disease Activity Index 2000 (SLEDAI-2K) and renal SLEDAI (rSLEDAI) scores were also completed to evaluate general and renal disease activity, respectively(11). Additionally, SLICC (Systemic Lupus International Collaborating Clinics/ American College of Rheumatology Damage Index) scores were obtained(12).

Plasma and serum samples were referred to the hospital's accredited central laboratory where biochemical parameters were analysed using standard methods. This included creatinine and complement (C3 and C4) performed on the Roche Cobas 6000, full blood count on the Sysmex XN-9000, and autoantibodies anti-double-stranded DNA (anti-dsDNA), anti-Smith (anti-Sm) and anti-nuclear antibodies (ANA) on the Thermo Scientific EliA Phadia 250. The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation(13). Aliquots of urine were used to qualitatively and quantitatively assess for blood and protein. Four further aliquots were centrifuged and stored at -80 degrees Celsius for the batched analysis of uMCP-1 and uTWEAK by ELISA methodology according to previously

described methods(14,15) and the relevant package inserts. Follow-up tests were performed at six-months and one-year.

Measurement of uMCP-1 and uTWEAK

uMCP-1 was measured using the Human MCP-1 Quantikine ELISA Kit by R&D Systems. This assay has a measuring range of 31.3 to 2000 picograms per millilitre (pg/mL) and performs with an inter-assay precision of 5.9%. uTWEAK was measured using the TWEAK Human Instant ELISA Kit by eBioscience (Thermo Fisher Scientific). This assay has a measuring range of 15.6 to 1000 pg/mL and performs with an inter-assay precision of 7.9%. Samples with analyte concentrations above the measuring range were repeated in dilution. Both uMCP-1 and uTWEAK results were also mathematically normalised to the urine creatinine (uCr) concentration. These results were denoted as MCP-1/Cr and TWEAK/Cr and expressed as picograms per milligram of creatinine (pg/mg Cr).

Renal Biopsy

All patients in the active group underwent biopsy at enrolment into the study and their histology results were interpreted by anatomical pathologists at our centre using the International Society of Nephrology/ Renal Pathology Society Classification(16).

Definition of Remission

Remission status was described as complete, partial or no remission. Complete remission (CR) in LN was defined as return of serum creatinine to previous baseline, plus a decline in the UPCR to <0.050 g/mmol. Partial remission (PR) was defined as stabilisation (±25%), or improvement of serum creatinine, but not to normal, plus a ≥50% decrease in UPCR. Alternatively, if there was nephrotic-range proteinuria then improvement required a ≥50%

reduction in UPCR, and a UPCR <0.300 g/mmol. Patients not meeting the above criteria were considered to have no remission (NR)(17).

Statistical Analysis

Statistical analysis was performed using Stata (StataCorp LLC). The data were summarised as means and standard deviations (mean [±SD]), or medians and interquartile ranges (median [p25–p75]), depending on whether the data were normally distributed or non-parametric, respectively. Categorical variables were summarised with frequencies and percentages. The data were tested for normality using the Shapiro-Wilk test, and most data found to be distributed non-parametrically. Groups were compared using Wilcoxon rank-sum tests for non-parametric data, or if normally distributed, the student t-test. Variables were assessed using Spearman correlation or linear regression models. Receiver operating characteristic (ROC) curves were generated for diagnostic test evaluation to assess the sensitivity and specificity pair corresponding to a particular decision threshold for disease activity and to measure how well the biomarkers performed in distinguishing between active and quiescent patients by area under the curve (AUC). A p-value <0.05 was deemed statistically significant.

Results

Baseline Characteristics of the Study Population

The baseline characteristics of the study population are presented in *Table 1*. Overall, there was a preponderance of females (76%) of mixed ancestry (64%) and the median age of all participants was 36 years. The active group (n=20) was both significantly younger and had a shorter duration of SLE. The histological diagnoses of the active group were class II (n=2), class III (n=3), class IV (n=9), class V (n=4), class III+IV (n=1) and class IV+V (n=1). Most active

participants therefore had proliferative LN (defined as classes III and IV, with or without class V) (n=14, 70%). Half of the patients demonstrated the presence of interstitial fibrosis in their biopsy report.

Baseline and Follow-up Clinical and Laboratory Findings

The patients' LN disease scores (SLEDAI-2K, rSLEDAI and SLICC) are presented in *Table 2*. At baseline, the active group SLEDAI-2K ($p<0.0001$), rSLEDAI ($p<0.0001$) and SLICC ($p<0.0001$) scores were higher than the quiescent group. At six-months, SLEDAI-2K scores remained increased in the active group ($p=0.001$), whereas rSLEDAI ($p=0.41$) and SLICC ($p=0.07$) scores were not different between the two groups. At one-year, both SLEDAI-2K ($p<0.001$) and rSLEDAI ($p=0.01$) scores were higher in the active group. Serum creatinine (SCr) and eGFR values were similar between the 2 groups throughout the study (*Table 2*). Both ANA ($p=0.002$) and ds-DNA ($p=0.002$) were significantly higher in the active group at baseline. Complements C3 ($p<0.001$) and C4 ($p<0.001$) were lower in the active versus the quiescent group at baseline, but not at follow-up. Qualitative and quantitative assessment of blood and protein in the urine were significantly higher in the active group at baseline compared to the quiescent group. Additional laboratory results are shown in Table 2.

uMCP-1 and uTWEAK Results

The results for the novel biomarkers MCP-1 and TWEAK are presented as absolute values in pg/mL (uMCP-1 and uTWEAK) and as values normalised to uCr concentration in pg/mg Cr (MCP-1/Cr and TWEAK/Cr) (Table 2). uMCP-1 (1440 [683–2729] vs 256 [175–477] pg/mL, $p<0.0001$) and uTWEAK (209 [117–312] vs 74 [11–173] pg/mL, $p=0.0008$) were increased in the active group when compared with the quiescent group. At six-month and one-year follow-up there was no significant difference in the results of these biomarkers between the two

groups (see Figure 1). When normalised for uCr the difference between the groups at baseline persisted (MCP-1/Cr 1093 [577–2014] vs 286 [138–774] pg/mg Cr, p<0.001 and TWEAK/Cr 159 [89–296] vs 63 [26–160] pg/mg Cr, p=0.02). At follow-up these biomarkers were similar between the groups.

Diagnostic Accuracy in Determining Disease Activity

The baseline results were then used to generate ROC curves as a method of assessing the diagnostic accuracy of the biomarkers in determining the active from the quiescent patients using their assigned disease status (group) as the comparator (*Figure 2 a–d*). The specified cut-off demonstrates the optimal combination of sensitivity and specificity for the analyte. The area under the curve (AUC) parameter illustrates how well the biomarkers performed in distinguishing between active and quiescent patients. At a cut-off of 462 pg/mL for uMCP-1, we obtained a sensitivity of 95% and specificity of 73% with an area under the curve (AUC) of 0.875 (95% CI=0.751–0.952, p<0.001). For MCP-1/Cr, a cut-off of 380 pg/mg Cr provided a sensitivity of 90% and specificity of 63% (AUC 0.803, 95% CI=0.667–0.902, p<0.0001). At a cut-off of 193 pg/mL for uTWEAK, a sensitivity of 60% and specificity of 90% was obtained (AUC 0.783, 95% CI=0.644–0.887, p<0.0001). For TWEAK/Cr, a cut-off of 71 pg/mg Cr had a sensitivity of 85% and specificity of 53% (AUC 0.689, 95% CI=0.0.543–0.812, p=0.013).

Correlation Throughout Study

Further analyses were performed using Spearman's rank test to evaluate the correlation between biomarker levels and clinical scores, throughout the study, and is shown in Supplementary Table 1. uMCP-1, uTWEAK, MCP-1/Cr and TWEAK/Cr showed significant correlation with each other, disease activity scores (SLEDAI-2K, rSLEDAI, SLICC), remission status, autoantibodies (ANA and dsDNA), dipsticks haematuria and proteinuria, and UPCR.

Remission Status

Supplementary Table 2 and Supplementary Figure 1 show the remission status of the patients. At baseline, all participants in the active group were NR. In the quiescent group, 10% were NR, 37% were PR and 53% were CR. In the active group at six-months, 11 participants had improved, and 2 remained NR. In the quiescent group, 72% remained the same, however 5 patients had worsened. In the active group at one-year, when compared to their status at their previous visit, 3 had improved, 7 remained the same, and 7 had worsened. In the quiescent group, 4 had improved, 13 remained the same, and 3 had worsened.

Table 3 shows a summary of both biomarker levels by remission status. uMCP-1 and MCP/Cr results differentiated the three remission status groups from one another (NR, PR and CR), whereas uTWEAK and TWEAK/Cr results were able to differentiate NR from PR and CR, but not PR from CR.

Discussion

Interest in identifying and validating useful biomarkers for LN persists. The main findings of our study include: (i) significant differences in both biomarker levels between patients with active and quiescent LN, suggesting the usefulness of these biomarkers in disease activity monitoring; (ii) both urinary biomarkers correlated with disease activity scores, various biochemical tests and with remission status throughout the study; (iii) uMCP-1 and uTWEAK performed better than values normalised to creatinine in terms of diagnostic accuracy on ROC curve analysis.

A pathogenic role for MCP-1 in the initiation and progression of LN has been characterised. In animal models, MCP-1 antagonism or gene therapy ameliorated these effects and MCP-1

knockout mice were less prone to LN(18–20). In humans, when compared to a wide range of cytokines, uMCP-1 performed better as a marker of disease activity in SLE, was reported to correlate with LN disease activity and seemed to have the potential to predict renal flares(21,22). In children with SLE, uMCP-1 performed well as a biomarker for LN, was increased in active vs inactive LN (or SLE without LN) and SLE vs controls, while correlating with SLEDAI scores and biochemical measures of disease status(23). In adults, a meta-analysis that included eight studies with 399 patients showed that uMCP-1 was consistently elevated in active vs quiescent LN (or control participants)(24). Therefore, uMCP-1 has proven to be a biomarker in LN with great potential(21), although further studies are still needed in different population groups to demonstrate clinical benefit.

Activated monocytes and macrophages infiltrate the kidneys in the pathogenesis of LN and are a major source of TWEAK(8). TWEAK effects downstream expression of inflammatory messengers, including MCP-1, in human mesangial cells, podocytes, and tubular cells(8,25). In early multicentre studies of SLE patients by Schwartz and colleagues, uTWEAK higher in those with LN, than in control groups without (overall p=0.039; SLE non-LN p=0.005; healthy control p=0.003; rheumatoid arthritis p=0.013). Additionally, they found uTWEAK levels would peak during a LN flare, and decrease after the flare, indicating its potential usefulness as a marker of LN disease activity(15,26). Similarly, the active group of our study had high levels of uTWEAK at baseline, the period in which they were experiencing a renal flare, along with increased levels of uMCP-1. More recently, studies in Egypt(27–30), China(31,32), Colombia(33), Korea(34), Mexico(35) and Thailand(36) have confirmed uTWEAK's potential as a biomarker in diverse populations of LN. A 2017 meta-analysis that included eight of these studies confirmed uTWEAK to be elevated in patients with active LN vs quiescent LN (p=0.006)(37).

Alharazy et al performed a study in a Malaysian LN population in which they assessed uMCP-1 levels at baseline, two-months and four-months, and found uMCP-1 to be higher in the active group and in participants who relapsed. In response to treatment, and especially in those who achieved remission, uMCP-1 progressively decreased from baseline until the end of the study(38). These trends were mirrored in our study in most indicators of LN disease activity, however SLEDAI-2K, remained different between the groups at both follow-ups. At one-year, rSLEDAI was again different between the groups and TWEAK/Cr was borderline for significance. These findings are compelling in view of the increase in the number of participants with a no remission status (9 in the active group at one-year vs only 2 at 6-months – *Supplementary Table 2*). While LN is a disease known to relapse and remit, both biomarkers maintained correlation with remission status throughout the study and showed higher strength of correlation than many standard measures of disease activity.

It is uncertain why the TWEAK/Cr results were borderline for significance at one year. An explanation could be that the active patients were now on maintenance therapy and therefore, less potent treatment regimens, with an overall re-emergence of the underlying inflammatory pathways. Urine protein and blood had also increased at one-year, while Hb had decreased, in support of this explanation. The UPCR at this time point was, however, not different between the two groups.

Alharazy et al described the remission status of their patients in their longitudinal MCP-1 study(38). They found a difference in uMCP-1 levels between patients with CR, or PR, when compared with NR, or those that relapsed (at baseline $p=0.002$, two-months $p<0.001$, four-months $p<0.001$). uMCP-1 levels were highest in patients with relapse, followed by no remission and lastly CR or PR. In our study, uMCP-1 levels (and MCP-1/Cr) were different in

the NR, PR and CR groups defined throughout the study. TWEAK levels have also been described in terms of remission status and are consistent with our findings. Suttichet et al, in a longitudinal multi-centre study, demonstrated that uTWEAK levels were persistently elevated in NR, trend down by 3 months in PR, and were consistently low in CR(36). Thus, uMCP-1 and TWEAK are able to identify the remission status of patients with LN and may be able to identify renal flare (*Supplementary Figure 1*).

ROC curve analysis in our study confirmed uMCP-1 to be a good test for determining disease activity in LN with an AUC of 0.875 (sensitivity 95%, specificity 73%). When normalized MCP-1/Cr performed less well than uMCP-1, however it was still above 80% and therefore a good test. uTWEAK had a higher specificity (90%) for disease activity as has been shown in a previous studies by Dong et al (specificity 93%, sensitivity 62%, AUC 0.81)(32) and Selim et al (90% specificity, 77.3% sensitivity, AUC 0.88)(30). This enhanced specificity has not been demonstrated in all studies of TWEAK(29,39) and was lost in our study when normalised as uTWEAK/Cr (specificity 53%, sensitivity 85%, AUC 0.689). The utility of normalising urine results to urine creatinine is discussed below.

uMCP-1, uTWEAK, MCP-1/Cr and TWEAK/Cr correlated significantly with one another throughout the study. With some exceptions, the novel biomarkers demonstrated correlation with the results of traditional measures of disease activity status, both positive (SLEDAI-2K, rSLEDAI, remission status, ANA, dsDNA, TSB, TSP and UPCR), and negative (C3 and C4). In a study by El-shehaby et al, urinary MCP-1 ($p<0.001$) and TWEAK ($p<0.001$) levels were increased in SLE patients with LN(27). Similar to our study, they found that rSLEDAI scores correlated positively with MCP-1 ($r=0.635$, $p<0.001$) and TWEAK ($r=0.612$, $p< 0.001$). MCP-1 also correlated with C3 ($r=–0.49$, $p<0.001$), and C4 ($r=–0.324$, $p=0.005$), while TWEAK

correlated with haematuria (r=0.254, p=0.03), C3 (r=–0.544, p<0.001) and C4 (r=–0.409, p<0.001). In other studies, TWEAK variably correlated with indices of disease activity such as SLEDAI, rSLEDAI, proteinuria, haematuria, C3, and C4(27,30,32,37,39).

Previous studies of uMCP-1 and uTWEAK have reported either absolute concentrations (pg/mL) or normalised to creatinine in the urine sample (pg/mg Cr). This calculation is traditionally performed to account for differences in hydration status of random urine samples, with UPCR being a widely accepted and utilised method of correction. We reported both and consider further discussion warranted as the two biomarkers generally performed better without normalisation as evidenced by ROC curve analysis. How urinary biomarker concentrations should be reported, and whether or not they should be normalised to urinary creatinine concentration, is still controversial(40). Implicit assumptions are made. The first assumption is that glomerular filtration accounts for the analyte's appearance in the urine. MCP-1 and TWEAK, however, mostly appear in the urine as a result of the local inflammatory environment, not filtration. Correcting for creatinine may therefore falsely assess the true activity of the inflammatory process occurring renally. Schwartz et al also did not find correlation between proteinuria and TWEAK/Cr levels, indicating that increased TWEAK in the urine is not reflective of a loss of the glomerular filtration barrier with subsequent overflow of protein into the urine(26). The second assumption is that a linear relationship exists for all patient results between the appearance of creatinine and the biomarker in the urine. We know, however, that patients with active disease processes are not likely to be in steady-state, whereas quiescent patients are. While normalisation may be useful and valid for the evaluation of chronic kidney disease, it may be inappropriate in acute disease states(40,41). The third assumption is that assays perform well irrespective of the urine sample's hydration status. The challenges of measuring biomarkers in urine with ELISA methodology have been

documented(42,43). Although most studies have found urine to be the matrix of choice in LN, a study in Korean females with SLE found that the serum level of TWEAK reflected both SLE and LN disease activity, whereas the urinary level of TWEAK, and the serum and urinary level of MCP-1, did not(34). In summary, the validity of normalisation of values is probably more appropriate in chronic rather than acute kidney conditions, and it is advisable that studies report both absolute and normalised values.

The strengths of the present study include: measurement of two novel urinary biomarkers longitudinally over one year, all participants had biopsy-proven LN (with the active arm's biopsy was around the time of enrollment), reporting of both absolute and normalized results, and the first study of both biomarkers in a Sub-Saharan population. The weaknesses of this study include: due to ethical and other considerations, renal biopsy was not performed on the quiescent group to exclude disease activity at enrollment, and there was a decrease in sample number at follow-up (especially in quiescent group).

uMCP-1 and uTWEAK reflect LN disease activity, and correlate with other standard of care biomarkers in a South African cohort, however, further studies are needed to assess additional clinical benefit. Nevertheless, the usefulness of these biomarkers may be enhanced by incorporating several biochemical markers into a panel for assessing LN disease activity.

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Declaration of Conflicting Interests

The Authors declare that there are no conflicts of interest

Tables and Figures

Table 1: Baseline Characteristics of Study Participants

Variable	Total n = 50	Active n = 20	Quiescent n = 30
Demographics			
- Age (years)	36 (27–41)	****26 (23–40)**	36 (30–41)
- Weight (kg)	65 (58–73)	60 (56–66)	68 (60–78)
- Female (n [%])	38 (76)	14 (70)	24 (80)
Ethnicity (n [%])			
- Black	16 (32)	7 (35)	9 (30)
- Mixed Ancestry	32 (64)	12 (60)	20 (67)
- Indian	1 (2)	0 (0)	1 (3)
- White	1 (2)	1 (5)	0 (0)
Duration of SLE (years)	8 (3–12)	***2 (1–7)**	10 (8–15)
Biopsy features (%)			
- Class II		10	
- Class III		15	
- Class IV		45	
- Class III + V		5	
- Class IV + V		5	
- Class V		20	
- Activity index		4 (2–9)	
- Chronicity index		2 (1–4)	

Results expressed as median (M) and interquartile ranges (IQR)- M(25–75). n - sample number; kg – kilogram. Class II – mesangial proliferative lupus glomerulonephritis (LGN); Class III – focal LGN; Class IV – diffuse segmental or global LGN; Class V – Membranous LGN. Proliferative LN includes classes III, IV, III+V and IV+V; Non-proliferative LN includes classes I, II and V). *Asterisks demonstrate significant difference between active and quiescent groups: * p<0.001; ** p<0.05*

Table 2: Laboratory Results and Clinical Scores at Baseline, Six-Months and One-Year

	BASELINE		SIX-MONTHS		ONE-YEAR	
VARIABLE	**Active**	**Quiescent**	**Active**	**Quiescent**	**Active**	**Quiescent**
Number	**n = 20**	**n = 30**	**n = 14**	**n = 18**	**n = 17**	**n = 19**
SLEDAI-2K	***17 (13–27)**	0 (0–0)	****4 (0–12)**	0 (0–0)	***8 (4–10)**	0 (0–2)
rSLEDAI	***8 (8–12)**	0 (0–4)	4 (0–4)	4 (0–4)	****4 (0–8)**	0 (0–0)
SLICC	***1.5 (1–2)**	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)
Blood						
Creatinine (umol/L)	104 (57–154)	76 (57–95)	91 (56–130)	74 (56–100)	76 (60–134)	81 (66–102)
eGFR (ml/min/1.73m^2)	64 (41–117)	96 (64–119)	91 (53–113)	102 (67–122)	81 (51–117)	82 (68–107)
ANA	***32 (22–40)**	9.7 (0.9–26)				
ds-DNA (IU/mL)	****248 (66.5–379)**	28.5 (4.8–86)	23 (12–109)	42 (3.4–90)	12 (8.6–61)	36 (5.1–75)
Anti-Sm (U/mL)	3.3 (0.9–75.7)	1.2 (0.7–3.4)	1.6 (0.7–9.5)	1.1 (0.8–2.3)	2.4 (0.6–72.9)	1.0 (0.6–2.9)
C3 (g/L)	***0.4 (±0.2)**	0.9 (±0.2)	1.0 (±0.3)	1.0 (±0.3)	1.0 (±0.4)	1.1 (±0.3)
C4 (g/L)	***0.09 (0.04–0.14)**	0.22 (0.16–0.25)	0.21 (0.14–0.23)	0.29 (0.24–0.32)	0.20 (0.15–0.31)	0.23 (0.18–0.33)
Hb (g/dL)	***9.2 (8.1–10)**	12.1 (10.9–13)	11.4 (11–12.1)	11.8 (10.4–13.4)	11.1 (10.2–12.3)	#12.3 (11.2–12.8)
WBC (x10^9/L)	5.9 (4.0–8.9)	6.0 (5.0–6.3)	7.4 (5.2–10.3)	5.2 (4.7–6.2)	4.5 (3.4–7.0)	5.4 (3.0–13.7)
Platelets (x10^9/L)	240 (±136)	295 (±109)	264 (±83)	264 (±68)	286 (±115)	268 (±65)
Urine						
TSP	***3 (2–3)**	1 (0–1)	2 (0–2)	0 (0–1)	****2 (1–2)**	0 (0–1)
TSB	***3 (1–3)**	0 (0–0)	0 (0–0)	0 (0–0)	*****0 (0–2)**	0 (0–0)
UPCR g/mmol Cr	***0.373 (0.175–0.558)**	0.039 (0.011–0.134)	0.068 (0.030–0.168)	0.067 (0.022–0.212)	0.078 (0.025–0.153)	0.027 (0.013–0.159)
Creatinine (mmol/L)	12.2 (±7.8)	10.0 (±5.7)	12.7 (±11.3)	12.6 (±4.9)	13.7 (±6.1)	14.8 (±8.1)
MCP-1 (pg/mL)	****1440 (683–2729)**	256 (175–477)	484 (217–830)	297 (131–686)	421 (203–1004)	363 (183–657)
MCP-1/Cr (pg/mgCr)	****1093 (577–2014)**	286 (138–774)	328 (241–648)	205 (115–397)	332 (211–484)	211 (105–473)
TWEAK (pg/mL)	****209 (117–312)**	74 (11–173)	42 (21–70)	27 (8–35)	65 (28–164)	40 (17–78)
TWEAK/Cr (pg/mgCr)	******159 (89–296)**	63 (26–160)	43 (15–106)	18 (6–32)	#53 (25–106)	22 (13–67)

Results expressed as M(25–75]) for non-parametric data or mean (X) and standard deviation X(±SD) - for parametric data. *eGFR – estimated glomerular filtration rate; TSP – test strip protein; TSB – test strip blood. A significant difference between the active and quiescent group is demonstrated in bold and symbols demonstrate the level of significance as follows: * p<0.001; ** p<0.05; *** p<0.01; # p=0.05*

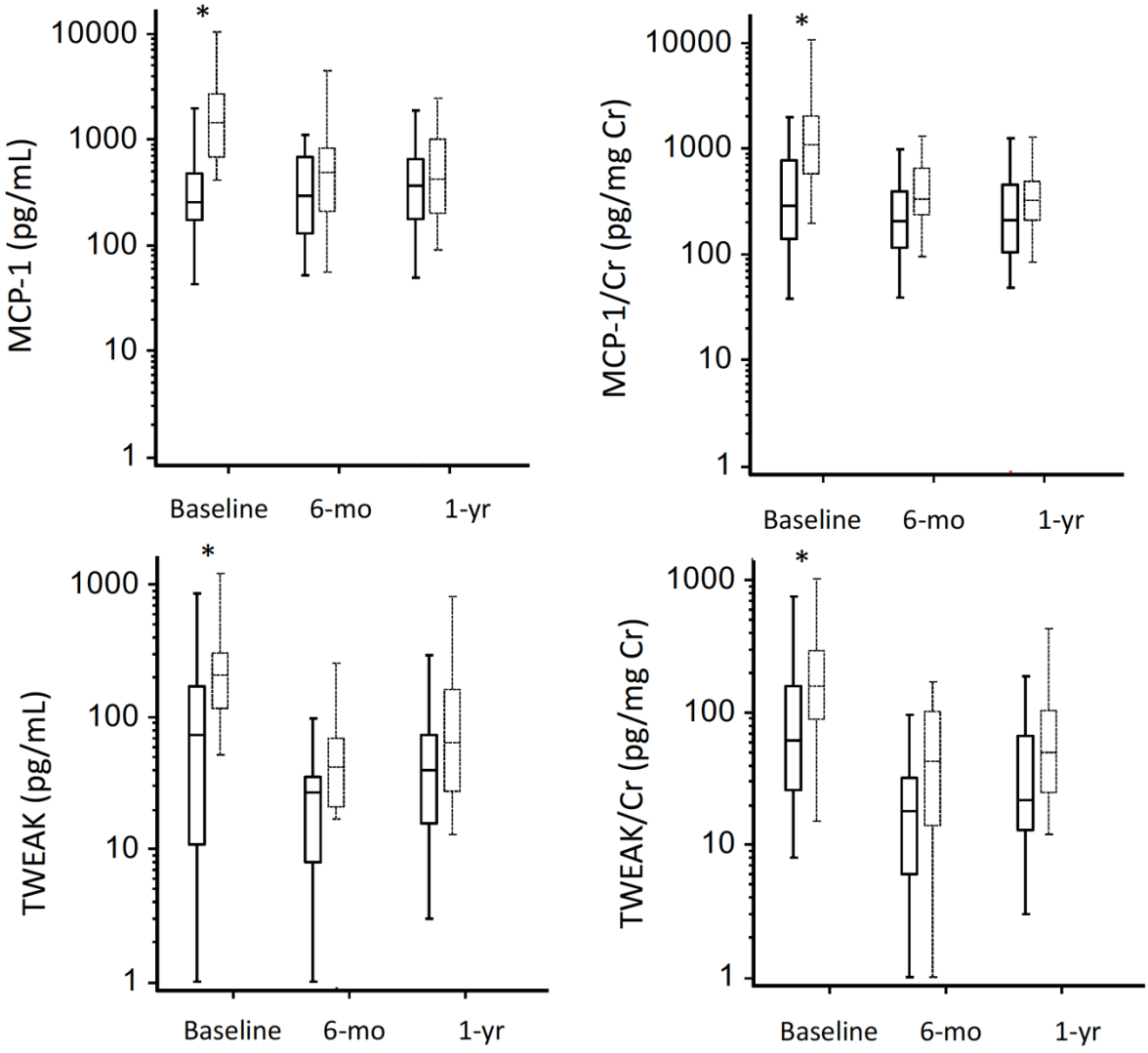


Figure 1: Longitudinal analysis of MCP-1 and TWEAK Concentrations

Box and whisker plots of MCP-1, MCP-1/Cr, Tweak and TWEAK/Cr at baseline, six-months and one-year. The inactive group is represented in bold on the left and the active group on the right (dashed line). Concentrations are depicted in log scale for clarity. Asterisk denotes that the results for the groups are different.

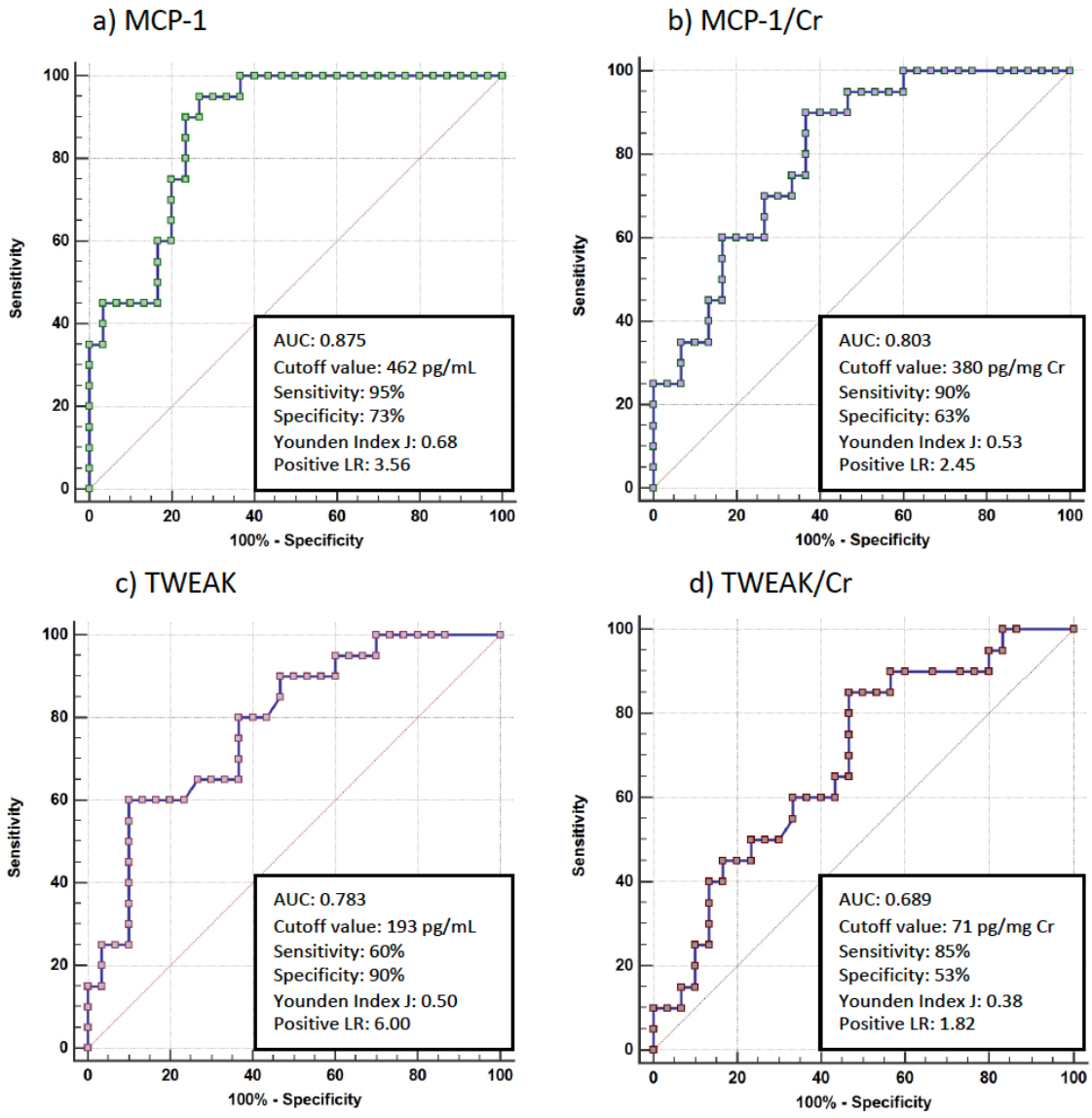


Figure 2 (a-d): Diagnostic Accuracy in Identifying Disease Activity

Receiver Operating Characteristic (ROC) Curves for the biomarkers MCP-1 and TWEAK, and normalised to urinary creatinine MCP-1/Cr and TWEAK/Cr, using results generated at baseline with activity status as the comparator. AUC – area under the curve

Supplementary Table 1: Spearman's Rank Correlations Between Biomarkers with rho (r) and p-values (p)

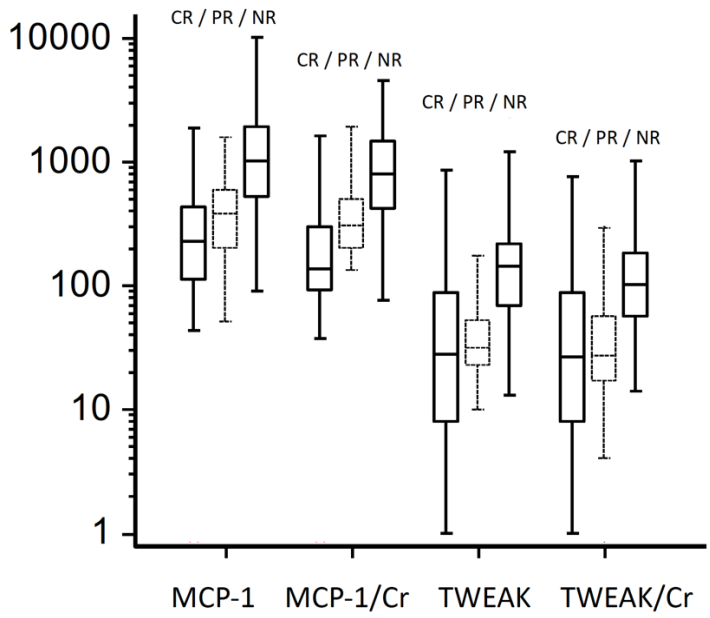
		uMCP1	uTWEAK	MCP1/Cr	TWEAK/Cr	SLEDAI	rSLEDAI	SLICC	RemStat	ANA	dsDNA	C3	C4	eGFR	Hb	TSB	TSP	UPCR
uMCP1	r		0.52	0.86	0.31	0.48	0.43	0.23	0.57	0.43	0.35	-0.37	-0.30	-0.25	-0.47	0.39	0.51	0.44
	p		<0.001	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	0.004	<0.001	<0.001	0.005	0.007	<0.001	<0.001	<0.001	<0.001
uTWEAK	r	0.52		0.51	0.88	0.41	0.39	0.33	0.46	0.53	0.32	-0.29	-0.27	-0.17	-0.41	0.40	0.51	0.38
	p	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.009	0.07	<0.001	<0.001	<0.001	<0.001
MCP1/Cr	r	0.86	0.51		0.51	0.45	0.48	0.30	0.60	0.41	0.40	-0.33	-0.28	-0.22	-0.51	0.36	0.49	0.44
	p	<0.001	<0.001		<0.001	<0.001	<0.001	0.001	<0.001	0.006	<0.001	0.001	0.009	0.01	<0.001	<0.001	<0.001	<0.001
TWEAK/Cr	r	0.31	0.88	0.51		0.33	0.37	0.34	0.40	0.41	0.31	-0.23	-0.18	-0.09	-0.38	0.34	0.41	0.33
	p	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001	0.006	<0.001	0.02	0.09	0.33	<0.001	<0.001	<0.001	<0.001
SLEDAI	r	0.48	0.41	0.45	0.33		0.68	0.35	0.59	0.57	0.35	-0.48	-0.49	-0.16	-0.32	0.57	0.63	0.49
	p	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.08	<0.001	<0.001	<0.001	<0.001
rSLEDAI	r	0.43	0.39	0.48	0.37	0.68		0.44	0.77	0.34	0.25	-0.29	-0.37	-0.26	-0.28	0.72	0.69	0.72
	p	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001	0.02	0.006	0.005	<0.001	0.004	0.002	<0.001	<0.001	<0.001
SLICC	r	0.23	0.33	0.30	0.34	0.35	0.44		0.39	0.27	0.21	-0.32	-0.16	-0.32	-0.32	0.42	0.32	0.36
	p	0.02	<0.001	0.001	<0.001	<0.001	<0.001		<0.001	0.08	0.03	0.003	0.1	<0.001	0.001	<0.001	<0.001	<0.001
RemStat	r	0.57	0.46	0.60	0.40	0.59	0.77	0.39		0.39	0.27	-0.35	-0.28	-0.31	-0.40	0.46	0.80	0.85
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		0.009	0.003	<0.001	0.008	<0.001	<0.001	<0.001	<0.001	<0.001
ANA	r	0.43	0.53	0.41	0.41	0.57	0.34	0.27	0.39		0.63	-0.69	-0.62	-0.13	-0.52	0.40	0.35	0.21
	p	0.004	<0.001	0.006	0.006	<0.001	0.02	0.08	0.009		<0.001	<0.001	<0.001	0.40	<0.001	0.006	0.02	0.1
dsDNA	r	0.35	0.32	0.40	0.31	0.35	0.25	0.21	0.27	0.63		-0.72	-0.61	-0.02	-0.31	0.36	0.25	0.19
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	0.03	0.003	<0.001		<0.001	<0.001	0.8	<0.001	<0.001	0.007	0.04
C3	r	-0.37	-0.29	-0.33	-0.23	-0.48	-0.29	-0.32	-0.35	-0.69	-0.72		0.65	0.16	0.36	-0.39	-0.23	-0.23
	p	<0.001	0.005	0.001	0.02	<0.001	0.005	0.003	<0.001	<0.001	<0.001		<0.001	0.1	<0.001	<0.001	0.03	0.03
C4	r	-0.30	-0.27	-0.28	-0.18	-0.49	-0.37	-0.16	-0.28	-0.62	-0.61	0.65		-0.03	0.21	-0.32	-0.26	-0.31
	p	0.005	0.009	0.009	0.09	<0.001	<0.001	0.1	0.008	<0.001	<0.001	<0.001		0.7	0.049	0.002	0.01	0.003
eGFR	r	-0.25	-0.17	-0.22	-0.09	-0.16	-0.26	-0.32	-0.31	-0.13	-0.02	0.16	-0.03		0.27	-0.23	-0.17	-0.00
	p	0.007	0.07	0.01	0.33	0.08	0.004	<0.001	<0.001	0.40	0.8	0.1	0.7		0.002	0.01	0.06	0.9
Hb	r	-0.47	-0.41	-0.51	-0.38	-0.32	-0.28	-0.32	-0.40	-0.52	-0.31	0.36	0.21	0.27		-0.27	-0.27	-0.23
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.001	<0.001	<0.001	<0.001	<0.001	0.049	0.002		0.003	0.003	0.01
TSB	r	0.39	0.40	0.36	0.34	0.57	0.72	0.42	0.46	0.40	0.36	-0.39	-0.32	-0.23	-0.27		0.47	0.36
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	<0.001	0.002	0.01	0.003		<0.001	<0.001
TSP	r	0.51	0.51	0.49	0.41	0.63	0.69	0.32	0.80	0.35	0.25	-0.23	-0.26	-0.17	-0.27	0.47		0.76
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.02	0.007	0.03	0.01	0.06	0.003	<0.001		<0.001
UPCR	r	0.44	0.38	0.44	0.33	0.49	0.72	0.36	0.85	0.21	0.19	-0.23	-0.31	-0.00	-0.23	0.36	0.76	
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.1	0.043	0.03	0.003	0.9	0.01	<0.001	<0.001	

Spearman's rank correlation with rho (r) and p-value for significance (p). Significant results are highlighted in black; non-significant results are grey. Rho values can be categorises as follows: 0.0-0.19 "very weak", 0.20-0.39 "weak", 0.40-.59 "moderate", 0.60-0.79 "strong", 0.80-1.0 "very strong.

Supplementary Table 2: Remission Status at Baseline and Follow-up

	Active	Quiescent
Baseline	*n=20*	*n=30*
NR	20(100)	3(10)
PR	0(0)	11(37)
CR	0(0)	16(53)
6-Months	*n=13*	*n=18*
NR	2(15)	4(22)
PR	6(46)	7(39)
CR	5(38)	7(39)
Improved	11(85)	0(0)
Same	2(15)	13(72)
Worsened	0(0)	5(28)
1-Year	*n=17*	*n=20*
NR	9(53)	6(30)
PR	5(29)	2(10)
CR	3(18)	12(60)
Improved	3(18)	4(20)
Same	7(41)	13(65)
Worsened	7(41)	3(15)

Remission status (no remission [NR], partial remission [PR] and complete remission [CR]) is shown here for the active and quiescent groups as number (percentage) in the group. Whether the patients had improved, stayed the same or worsened, according to their remission status, is shown numerically as number of participants (percentage) in the group.



Supplementary Figure 1: Biomarker Concentrations as a Function of Remission Status

Box and whisker plots demonstrating the concentrations of MCP-1, MCP/Cr, TWEAK and TWEAK/Cr when plotted according to complete remission (CR), partial remission (PR) and no remission (NR).

Table 3: Remission Status

	Remission Status		
	None	*Partial*	*Complete*
uMCP-1	1020 (530-1951)	387 (203-606)	230 (113-433)
Partial	p<0.001		
Complete	p<0.001	p=0.04	
uTWEAK	145 (70-221)	32 (23-53)	28 (8-89)
Partial	p<0.001		
Complete	p<0.001	p=0.6	
MCP/Cr	795 (426 -1496)	310 (203-505)	139 (94-301)
Partial	p<0.001		
Complete	p<0.001	<0.001	
TWEAK/Cr	102 (58-183)	27 (17-58)	27 (8-88)
Partial	p<0.001		
Complete	p<0.001	p=0.5	

Biomarker results according to no, partial or complete remission status (see text for definitions). The Mann-Whitney test was used to compare the biomarker values for each remission status group (with significant p values in black and non-significant p-values in grey).

References

1. Wu H, Zeng J, Yin J, Peng Q, Zhao M, Lu Q. Organ-specific biomarkers in lupus. Autoimmun Rev. 2017;16(4):391–7.

2. Mok CC. Biomarkers for Lupus Nephritis: A Critical Appraisal. J Biomed Biotechnol. 2010;

3. Li Y, Fang X, Li QZ. Biomarker Profiling for Lupus Nephritis. Genomics, Proteomics Bioinforma [Internet]. 2013;11(3):158–65. Available from: http://dx.doi.org/10.1016/j.gpb.2013.05.003

4. Michaelson JS, Wisniacki N, Burkly LC, Putterman C. Role of TWEAK in lupus nephritis: A bench-to-bedside review. Journal of Autoimmunity. 2012.

5. Kelley VR, Rovin BH. Chemokines: Therapeutic targets for autoimmune and inflammatory renal disease. Springer Seminars in Immunopathology. 2003.

6. Rodríguez-Castro S, Rosetti F, Flores-Mendoza G, Sansón SP, Crispín JC. Mechanisms of Tissue Injury in Lupus Nephritis. Trends Mol Med. 2018;24(4):364–78.

7. Yadav A, Saini V, Arora S. MCP-1: Chemoattractant with a role beyond immunity: A review. Clinica Chimica Acta. 2010.

8. Browning B, Schiffer L, Campbell S, Putterman C, Zheng T, Berman JW, et al. Proinflammatory Effects of Tweak/Fn14 Interactions in Glomerular Mesangial Cells. J Immunol. 2014;176(3):1889–98.

9. Xia Y, Herlitz LC, Gindea S, Wen J, Pawar RD, Misharin A, et al. Deficiency of Fibroblast Growth Factor-Inducible 14 (Fn14) Preserves the Filtration Barrier and Ameliorates Lupus Nephritis. J Am Soc Nephrol. 2014;

10. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis and rheumatism. 1997.

11. Gladman DD, Ibañez D, Urowltz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol. 2002;

12. Gladman DD, Urowitz MB. The SLICC/ACR damage index: Progress report and experience in the field. Lupus. 1999;8(8):632–7.

13. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro III AF, Feldman HI, et al. A New Equation to Estimate Glomerular Filtration Rate Disclosure of conflicts of interest: We have received confirmation from Drs. Ann Intern Med. 2009;150(9):604–12.

14. Wada T, Yokoyama H, Su SB, Mukaida N, Iwano M, Dohi K, et al. Monitoring urinary levels of monocyte chemotactic and activating factor reflects disease activity of lupus nephritis. Vol. 49, Kidney International. 1996. p. 761–7.

15. Schwartz N, Rubinstein T, Burkly LC, Collins CE, Blanco I, Su L, et al. Urinary TWEAK as a biomarker of lupus nephritis: A multicenter cohort study. Arthritis Res Ther. 2009;11(5):1–10.

16. Hill GS, Delahousse M, Nochy D, Tomkiewicz E, Remy P, Mignon F, et al. A new morphologic index for the evaluation of renal biopsies in lupus nephritis. Kidney Int. 2000;

17. Kidney Disease Improving Global Outcomes. KDIGO Clinical practice guideline for glomerulonephritis. Kidney Int Suppl Int Suppl. 2012;

18. Hasegawa H, Kohno M, Sasaki M, Inoue A, Ito MR, Terada M, et al. Antagonist of monocyte chemoattractant protein 1 ameliorates the initiation and progression of lupus nephritis and renal vasculitis in MRL/lpr mice. Arthritis Rheum. 2003;

19. Shimizu S, Nakashima H, Masutani K, Inoue Y, Miyake K, Akahoshi M, et al. Anti-monocyte chemoattractant protein-1 gene therapy attenuates nephritis in MRL/lpr mice. Rheumatology. 2004;

20. Tesch GH, Maifert S, Schwarting A, Rollins BJ, Kelley VR. Monocyte

Chemoattractant Protein 1–Dependent Leukocytic Infiltrates Are Responsible for Autoimmune Disease in Mrl- Fas lpr Mice . J Exp Med. 1999;

21. Barbado J, Martin D, Vega L, Almansa R, Gonçalves L, Nocito M, et al. MCP-1 in urine as biomarker of disease activity in Systemic Lupus Erythematosus. Cytokine [Internet]. 2012;60(2):583–6. Available from: http://dx.doi.org/10.1016/j.cyto.2012.07.009

22. Rovin BH, Zhang X. Biomarkers for lupus nephritis: The quest continues. Clinical Journal of the American Society of Nephrology. 2009.

23. El Hamshary A, Abd El Raheim Y, Mohamed A, Talaat A, Ghobrial E. Urinary monocyte chemoattractant protein-1 as a biomarker of lupus nephritis activity in children. Saudi J Kidney Dis Transplant. 2015;26(3):507.

24. Lee YH, Song GG. Urinary MCP-1 as a biomarker for lupus nephritis: a meta-analysis. Z Rheumatol [Internet]. 2017;76(4):357–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27278779

25. Chen J, Wei L, Xia Y. Roles of tumour necrosis factor-related weak inducer of apoptosis/fibroblast growth factor-inducible 14 pathway in lupus nephritis. Nephrology. 2017.

26. Schwartz N, Michaelson JS, Putterman C. Lipocalin-2, TWEAK, and other cytokines as urinary biomarkers for lupus nephritis. Ann N Y Acad Sci. 2007;1109:265–74.

27. El-Shehaby A, Darweesh H, El-Khatib M, Momtaz M, Marzouk S, El-Shaarawy N, et al. Correlations of urinary biomarkers, TNF-like weak inducer of apoptosis (TWEAK), osteoprotegerin (OPG), monocyte chemoattractant protein-1 (MCP-1), and IL-8 with lupus nephritis. J Clin Immunol. 2011;31(5):848–56.

28. Abd El-Fattah El-Feqi M, Mohamed RA, Salem MN, Taha HA, Eesa NN. Urinary TNF-like weak inducer of apoptosis (TWEAK) as a biomarker of lupus nephritisTNF-

ähnlicher schwacher Induktor von Apoptose (TWEAK) im Urin als Biomarker einer Lupusnephritis. Z Rheumatol. 2016;77(1):71–7.

29. Elsayed M, Elhefnawy KA, Qassem AA, Amr GE. Urinary Tumor Necrosis Factor-Like Weak Inducer of Apoptosis (TWEAK) As a Marker for Lupus Nephritis Activity. Int J Sci Res. 2013;4(2):1615–9.

30. Selim ZI, Khader TM, Mohammed HO, Al-Hammady DH, Seif HMA, Al-Johi AA. Urinary Tumor Necrosis Factor-Like Weak Inducer of Apoptosis (uTWEAK) as a biomarker for lupus nephritis activity and its correlation with histolopathological findings of renal biopsy. Egypt Rheumatol [Internet]. 2018; Available from: https://doi.org/10.1016/j.ejr.2018.01.008

31. Xuejing Z, Jiazhen T, Jun L, Xiangqing X, Shuguang Y, Fuyou L. Urinary TWEAK level as a marker of lupus nephritis activity in 46 cases. J Biomed Biotechnol. 2012;2012(March 2009):1–7.

32. Zhu P, Li ZQ, Zheng ZH, Dong XW, Ding J, Shi JH, et al. Combined detection of uMCP-1 and uTWEAK for rapid discrimination of severe lupus nephritis. Lupus. 2018;096120331875850.

33. Gómez-Puerta JA, Ortiz Reyes BL, Urrego T, Vanegas AL, Muñoz CH, Restrepo M, et al. A panel of urinary biomarkers to assess renal involvement in latin american patients with systemic lupus erythematosus. Arthritis Rheumatol [Internet]. 2016;68:158–60. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L613887196%5Cnhttp://dx.doi.org/10.1002/art.39977%5Cnhttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=23265205&id=doi:10.1002%2Fart.39977&atitle=A+panel+of+urinary+biomarkers+to+asse

34. Choe JY, Kim SK. Serum TWEAK as a biomarker for disease activity of systemic lupus erythematosus. Inflamm Res. 2016;65(6):479–88.

35. Reyes-Martínez F, Pérez-Navarro M, Rodríguez-Matías A, Soto-Abraham V, Gutierrez-Reyes G, Medina-Avila Z, et al. Assessment of urinary TWEAK levels in Mexican patients with untreated lupus nephritis: An exploratory study. Nefrologia [Internet]. 2018;38(2):152–60. Available from: http://dx.doi.org/10.1016/j.nefro.2017.04.005

36. Suttichet TB, Kittanamongkolchai W, Phromjeen C, Anutrakulchai S, Panaput T, Ingsathit A, et al. Urine TWEAK level as a biomarker for early response to treatment in active lupus nephritis: a prospective multicentre study. Lupus Sci Med [Internet]. 2019;6(1):e000298. Available from: http://lupus.bmj.com/lookup/doi/10.1136/lupus-2018-000298

37. Lee YH, Song GG. Urinary Tumor Necrosis Factor-like Weak Inducer of Apoptosis as a Biomarker for Lupus Nephritis: A Meta-analysis. J Rheum Dis [Internet]. 2017;24(2):85. Available from: https://synapse.koreamed.org/DOIx.php?id=10.4078/jrd.2017.24.2.85

38. Shah SA, Abdul Gafor AH, Kong NCT, Mohd M, Ba'in A, Alharazy S. Urine Monocyte Chemoattractant Protein-1 and Lupus Nephritis Disease Activity: Preliminary Report of a Prospective Longitudinal Study. Autoimmune Dis. 2015;2015:1–13.

39. Reyes-Martínez F, Pérez-Navarro M, Rodríguez-Matías A, Soto-Abraham V, Gutierrez-Reyes G, Medina-Avila Z, et al. Assessment of urinary TWEAK levels in Mexican patients with untreated lupus nephritis: An exploratory study. Nefrologia [Internet]. 2018;38(2):152–60. Available from: https://doi.org/10.1016/j.nefroe.2018.02.006

40. Waikar SS, Sabbisetti VS, Bonventre J V. Normalization of urinary biomarkers to creatinine during changes in glomerular filtration rate. Kidney Int [Internet]. 2010;78(5):486–94. Available from: http://dx.doi.org/10.1038/ki.2010.165

41. Tang KWA, Toh QC, Teo BW. Normalisation of urinary biomarkers to creatinine for

clinical practice and research – When and why. Singapore Med J. 2015;56(1):7–10.

42. Reid CN, Stevenson M, Abogunrin F, Ruddock MW, Emmert-Streib F, Lamont J V., et al. Standardization of Diagnostic Biomarker Concentrations in Urine: The Hematuria Caveat. PLoS One. 2012;7(12).

43. Lewis EC, Taylor TP, Oates JC, Janech MG, Arthur JM, Slate EH. Overcoming the Effects of Matrix Interference in the Measurement of Urine Protein Analytes. Biomark Insights. 2012;7:BMI.S8703.

Appendices

Appendix 1: Ethics Approval Letters

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492 • **Facsimile** [021] 406 6411
Email: Sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

02 July 2014

HREC/REF: 402/2014

Dr I Okpechi
Nephrology & Hypertension
E-13
Renal Unit
NGSH

Dear Dr Okpechi

Project Title: USING URINARY BIOMARKERS (MCP-1 and TWEAK) IN RISK PREDICTION MODELS TO ENHANCE AND IMPROVE DIAGNOSIS (HENCE INFLUENCING CHOICE OF THERAPY AND PATIENT OUTCOME) IN PATIENTS WITH LUPUS NEPHRITIS IN CAPE TOWN (A sub-study of the ALUGEN Registry)

Thank you submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year until the 30 July 2015.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

Hrec/ref:405/2014

HUMAN RESEARCH ETHICS COMMITTEE
03 JUL 2015
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/07/2016
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	Signature Removed	Date Signed	08/07/2015

08/07/2015 Please provide study staff amendment forms for the new study staff. Thank you TBurgess

Comments to PI from the HREC
Dear HREC, We got approval for this study last year but will only start recruitment later this year. No patient has been recruited thus far for the study.

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	3rd July 2015		
HREC REF Number	402/2014	Current Ethics Approval was granted until	30th July 2015
Protocol title	Using urinary biomarkers (MCP-1 and TWEAK) in risk prediction models to enhance and improve diagnosis (hence influencing choice of therapy and patient outcome) in patients with lupus nephritis in Cape Town (A sub-study of the ALUGEN Registry).		
Protocol number (if applicable)	NA		
Are there any sub-studies linked to this study?	☐ Yes ☐ No✓		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	**A/Prof Ikechi Okpechi**		
Department / Office Internal Mail Address	**E13 Renal Unit Groote Schuur Hospital, Observatory, 7925 Cape Town**		

1.1 Does this protocol receive US Federal funding?	☐ Yes	☐ No✓
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	☐ Yes	☐ No
1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	☐ Yes	☐ No

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

09 December 2016

HREC REF: 856/2016

A/Prof I Okpechi
Division of Nephrology & Hypertension
E-13-Renal Unit
GSH

Dear A/Prof Okpechi

PROJECT TITLE: USING URINARY BIOMARKERS (MCP-1 AND TWEAK) IN RISK PREDICTION MODELS TO ENCHANCE THE ASSESSMENT OF DISEASE ACTIVITY (therefore influencing choice of therapy and patient outcome) IN PATIENTS WITH LUPUS NEPHRITIS IN CAPE TOWN- (MMeD-candidate-Dr J Rusch) SUB-STUDY LINKED TO 402/2014

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 December 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student, Dr J Rusch will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

HUMAN RESEARCH ETHICS COMMITTEE
- 9 APR 2018
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30·04·2019
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	Signature Removed	Date Signed	09/04/2018

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	9th April 2018		
HREC REF Number	402/2014	Current Ethics Approval was granted until	13/Nov/2017
Protocol title	Using urinary biomarkers (MCP-1 and TWEAK) in risk prediction models to enhance and improve diagnosis (hence influencing choice of therapy and patient outcome) in patients with lupus nephritis in Cape Town (A sub-study of the ALUGEN Registry).		
Protocol number (if applicable)	NA		
Are there any sub-studies linked to this study?	☐ Yes ☐ No✓		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study			
Principal Investigator	Prof Ikechi Okpechi		
Department / Office Internal Mail Address	Medicine / E13 Renal Unit Groote Schuur Hospital, Observatory, 7925, Cape Town		

1.1 Does this protocol receive US Federal funding?	☐ Yes	☐ No✓

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix 2: Consent Forms

TWEAK STUDY (LUPUS BIOMARKER STUDY)

INFORMED CONSENT

Using urinary biomarkers (MCP-1 and TWEAK) in risk prediction models to enhance and improve diagnosis (hence influencing choice of therapy and patient outcome) in patients with lupus nephritis in Cape Town (A sub-study of the ALUGEN Registry).

I hereby confirm that the study doctor (**Dr. .....**) has informed me about the nature, conduct, benefits and risks of this study. I have also received, read, and understand the written Patient Information.

I am aware that the results of the study will be anonymously processed into a study report.

I may, at any stage, withdraw my consent and participation without prejudice.

I have had sufficient opportunity to ask questions and declare myself prepared to participate in the study.

Patient's Name	Patient's Signature	Date

Doctor's Name	Doctor's Signature	Date

INFORMED CONSENT FOR PARTICIPATION IN A STUDY ENTITLED: "Correlation between urinary biomarkers (uMCP-1 and uTWEAK), renal histology and early response to therapy in newly biopsied patients with lupus nephritis".

Study Number: e.g.	M	M	W	1	A	0	0	1

Study Aim:

The purpose of this study is to assess if there is any relationship between levels of particles called Tumor Necrotic Factor-like weak inducer of apoptosis (TWEAK) and monocyte chemoattractant protein-1 (MCP-1) in urine and the kidney biopsy findings in patients with systemic lupus erythematosus (SLE) the involves the kidneys (Lupus Nephritis) and whether the levels of these particles can be used to determine whether patients will respond to treatment after 6 months from the time of kidney biopsy. The results of this study will be used to guide doctors treating patients with lupus nephritis on whether they can treat patients with this disease without having to a kidney biopsy while basing the decision only on the urine levels of the particles named above. There are studies that have been done that show that there could be a relationship between the severity of this disease and the levels in urine of these particles.

Involvement:

You will be asked, by consenting, allow the study person to collect your blood and urine to test them for these particles. You will also be asked to come back after 6 months of treatment and have the levels these particles to be re-measured. The samples collected will be stored in a fridge at -80 degrees celcius until a point when all the collected samples will be measures.

Confidentiality:

Any information that is obtained in connection with this study and that can be identified with you will remain strictly confidential. However, the data may be seen by the ethical review committee and may be published without disclosing your identity.

Withdrawal from the study:

Your participation in this study is voluntary; you therefore have a right to withdraw from the study anytime. In case of withdrawal your information will be destroyed and will not be used as part of the data

Risks and Benefits:

There are no risks involved in participating in the study. At each visit during the study you will be compensated with R50 which you are expected to use for transportation to the study site. However, the results from this study will help us to determine whether urinary TWEAK and MCP-1 correlate with kidney biopsy results and response to treatment.

Contacts:

If you have questions regarding your rights as a research participant, you may wish to contact the principal investigators: A/Prof. Ikechi Okpechi and Dr Mothusi Walter Moloi, University of Cape Town, E13, Division of Nephrology and Hypertension, Groote Schuur Hospital (Tel: +27 214043310)

The study has been approved by the Human Research and Ethics Committee, Faculty of Health Sciences, University of Cape Town. If you have further questions on your rights as a study participant you may consult: Human Research Ethics Committee, E53 Room 46, Old Main Building, Groote Schuur Hospital, (Tel: +27 21 6501236)

Written Consent / Agreement

I..... (name of the patient) being ..... years old (with a sound mind to consent) have been informed about the study entitled: **"Correlation between urinary biomarkers (uMCP-1 and uTWEAK), renal histology and early response to therapy in newly biopsied patients with lupus nephritis"**. I have been given the opportunity to ask questions concerning the study and these have been answered to my satisfaction. I understand that I may at any time during the study revoke my consent and withdrawal from the study without any penalty. I also understand that giving consent to enrol into the study does not take away my legal rights in case of negligence or other legal fault by anyone who is involved in the study. I therefore volunteer to participate in this study

Signature of participant or thumb print.....Date ----------

Signature of witness .....Date ----------

Name of the patient obtaining consent.....

Signature of the person obtaining consent: .....Date ----------

Appendix 3: ALUGEN Registry Summary

ALUGEN Registry Summary:

Informed Consent	√
Disease History	Symptom onset, ethnic group (patient self-reported), family history of connective tissue disease
Socio-Demographics	Level of education, employment status, marital status, household ownership, type and annual income
Details of Organ Involvement	Constitutional symptoms, renal, skin, musculoskeletal, pulmonary, cardiovascular, neuropsychiatric, obstetric, ocular,vascular, haemotological, gastrointestinal immunological
Medication	Details of oral and intravenous corticosteroids, immunosuppressants, and all other medication
Comorbidities	Hypertension, diabetes, FMS, thyroid disorders, cardiovascular events, malignancy, osteoporosis, infection including TB, hepatitis B and C, HIV
Anthropometric Details	Weight, height, waist and hip circumference
Disease Activity	SLEDAI-2K
Damage	SLICC/ACR Damage Index
Blood tests	FBC, creatinine, autoantibodies, complement, lipogram
Urine tests	Protein : creatinine ratio
Fatigue	FACIT-Fatigue Scale
Health-related Quality of Life	SF36 Survey
Serious Adverse Event Log	Death or hospitalisation

Appendix 4: Data Sheets

TWEAK STUDY (LUPUS BIOMARKER STUDY)

ALUGEN

INSERT PATIENT STICKER HERE

STUDY NUMBER					
VISIT 1	**VISIT DATE:**				
DOB	DD/MM/YYYY	Study arm	ACTIVE / QUIESCENT		
GENDER	M / F	SLEDAI SCORE			
ETHNICITY	B / W / C / I / A	SLICC SCORE			
SMOKING	Ex / Y / N	Urinalysis	Protein	RBC	
ALCOHOL USE	Ex / Y / N		WBC	Nitrite	
OCCUPATION	Student / Employed/ Unemployed/	Urine microscopy	RBC casts	WBC casts	
YEARS OF EDUCATION	0 – 25 (----------)		Granular casts	Hyaline casts	
HIGHEST QUALIFICATION	Pry / Sec / Tertiary / Post-grad	FBC			
DATE OF SLE DIAGNOSIS		ANA			
NUMBER OF KIDNEY BIOPSIES		Anti-Ds-DNA			
DATE LAST KIDNEY BIOPSY		Anti-Sm			
LN – CLASS (MOST RECENT)		C3			
WEIGHT (KG)		C4			
HEIGHT (M)		UPCR			
SBP (MMHG)		Creatinine			
DBP (MMHG)		TWEAK			
CURRENT REMISSION STATUS	CR / PR / NR	MCP-1			
CURRENT MEDICATIONS	CQ	STEROIDS	CYC	MMF	AZA

TWEAK STUDY (LUPUS BIOMARKER STUDY)

INSERT PATIENT STICKER HERE

Study number					
VISIT 2	**VISIT DATE:**				
WEIGHT (kg)					
SBP (mmHg)					
DBP (mmHg)					
STUDY ARM	ACTIVE / QUIESCENT				
SLEDAI SCORE					
SLICC SCORE					
URINALYSIS	PROTEIN		RBC		
	WBC		NITRITE		
URINE MICROSCOPY	RBC CASTS		WBC CASTS		
	GRANULAR CASTS		HYALINE CASTS		
FBC					
Anti-Ds-DNA					
Anti-Sm					
C3					
C4					
UPCR					
SERUM CREATININE					
U-TWEAK					
U-MCP-1					
CURRENT REMISSION STATUS	CR / PR / NR				
CURRENT MEDICATIONS	CQ	STEROIDS	CYC	MMF	AZA

TWEAK STUDY (LUPUS BIOMARKER STUDY)

INSERT PATIENT STICKER HERE

Study number					
VISIT 3	**VISIT DATE:**				
WEIGHT (kg)					
SBP (mmHg)					
DBP (mmHg)					
STUDY ARM	ACTIVE / QUIESCENT				
SLEDAI SCORE					
SLICC SCORE					
URINALYSIS	PROTEIN		RBC		
	WBC		NITRITE		
URINE MICROSCOPY	RBC CASTS		WBC CASTS		
	GRANULAR CASTS		HYALINE CASTS		
FBC					
Anti-Ds-DNA					
Anti-Sm					
C3					
C4					
UPCR					
SERUM CREATININE					
U-TWEAK					
U-MCP-1					
CURRENT REMISSION STATUS	CR / PR / NR				
CURRENT MEDICATIONS	CQ	STEROIDS	CYC	MMF	AZA

Appendix 5: SLEDAI-2K disease activity questionnaire

SLEDAI-2K disease activity questionnaire:

For an item to be scored the indicated weight, the manifestation must have been **Present in the past 10 days.**

Weight	Tick if present	Descriptor	Definition
8		Seizure	Recent onset, exclude metabolic, infectious or drug causes.
8		Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Exclude uremia and drug causes
8		Organic brain syndrome	Altered mental function with impaired orientation, memory, or other intellectual function, with rapid onset and fluctuating clinical features, inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious, or drug causes.
8		Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid, or optic neuritis. Exclude hypertension, infection, or drug causes.
8		Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8		Lupus headache	Severe, persistent headache; may be migrainous, but must be nonresponsive to narcotic analgesia.
8		CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8		Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4		Arthritis	$\geq$ 2 joints with pain and signs of inflammation (i.e., tenderness, swelling or effusion).
4		Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
4		Urinary casts	Heme-granular or red blood cell casts.
4		Hematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4		Proteinuria	>0.5 gram/24 hours
4		Pyuria	>5 white blood cells/high power field. Exclude infection.
2		Rash	Inflammatory type rash.
2		Alopecia	Abnormal, patchy or diffuse loss of hair.
2		Mucosal ulcers	Oral or nasal ulcerations.
2		Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2		Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.
2		Low complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory
2		Increased DNA binding	Increased DNA binding by Farr assay above normal range for testing laboratory.
1		Fever	>38° C. Exclude infectious cause.
1		Thrombocytopenia	<100,000 platelets / x10^9/L, exclude drug causes.
1		Leukopenia	< 3,000 white blood cells / x10^9/L, exclude drug causes.

TOTAL SCORE: _____

Physician Global Activity Assessment:

No Activity	Mild Activity	Moderate Activity	Severe Activity

TWEAK-MCP-1 STUDY

Appendix 6: SLICC Scoring sheet

Systemic Lupus International Collaborating Clinics Damage Index

(Circle the appropriate score)

ITEM	0	1	2	3
Ocular (either eye, by clinical assessment)				
- Any cataract ever	0	1		
- Retinal change or optic atrophy	0	1		
Neuropsychiatric				
- Cognitive impairment (e.g. memory deficit, poor calculation etc)	0	1		
- Cerebrovascular accident (score 2 if >1)	0	1	2	
- Cranial or peripheral neuropathy (excluding optic)	0	1		
- Transverse myelitis	0	1		
Renal				
- Estimated or measured glomerular filtration rate< 50%	0	1		
- Proteinuria >3.5 gm/24hours or UPCR >0.35g/mmol	0	1		
- End-stage renal disease (regardless of dialysis or transplantation	0			3
Pulmonary				
- Pulmonary hypertension (right ventricular prominence, or loud P2	0	1		
- Pulmonary fibrosis (physical and radiograph)	0	1		
- Shrinking lung (radiograph)	0	1		
- Pleural fibrosis (radiograph)	0	1		
- Pulmonary infarction (radiograph)	0	1		
Cardiovascular				
- Angina or coronary artery bypass	0	1		
- Myocardial infarction ever (score 2 if > 1)	0	1	2	
- Cardiomyopathy (ventricular dysfunction)	0	1		
- Valvular disease (diastolic murmur, or systolic murmur >3/6)	0	1		
- Pericarditis for 6 months, or pericardiectomy	0	1		
Peripheral vascular				
- Claudication for 6 months	0	1		
- Minor tissue loss (pulp space)	0	1		
- Significant tissue loss ever (e.g. loss of digit or limb)(score 2 if > 1 site)	0	1	2	
- Venous thrombosis with swelling, ulceration, or venous stasis	0	1		
Gastrointestinal				
- Mesenteric insufficiency	0	1		
- Infarction or resection of bowel below duodenum spleen, liver, or gall bladder ever (score 2 if > 1 site)	0	1	2	
- Chronic peritonitis	0	1		
- Stricture or upper gastrointestinal tract surgery ever	0	1		
Musculoskeletal				
- Muscle atrophy or weakness	0	1		
- Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	0	1		
- Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	0	1		
- Avascular necrosis (score 2 if > 1)	0	1	2	
- Osteomyelitis	0	1		
Skin				
- Scarring chronic alopecia	0	1		
- Extensive scarring or panniculum other than scalp and pulp space	0	1		
- Skin ulceration (excluding thrombosis) for > 6 months	0	1		
Premature gonadal failure	0	1		
Diabetes regardless of treatment	0	1		
Malignancy (exclude dysplasia) (score 2 if > 1site)	0	1	2	

TWEAK/MCP-1 STUDY

Appendix 7: LUPUS - Journal Guidelines to Authors of Research Articles

Accessed: https://journals.sagepub.com/author-instructions/LUP

Manuscript Submission Guidelines: *Lupus*

This Journal is a member of the Committee on Publication Ethics.

This Journal recommends that authors follow the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals formulated by the International Committee of Medical Journal Editors (ICMJE).

Only manuscripts of sufficient quality that meet the aims and scope of *Lupus* will be reviewed.

There are no fees payable to submit or publish in this journal.

As part of the submission process you will be required to warrant that you are submitting your original work, that you have the rights in the work, that you are submitting the work for first publication in the Journal and that it is not being considered for publication elsewhere and has not already been published elsewhere, and that you have obtained and can supply all necessary permissions for the reproduction of any copyright works not owned by you.

1. What do we publish?

1.1 Aims & Scope

The only fully peer-reviewed international journal devoted exclusively to lupus (and related disease) research.

Lupus includes the most promising new clinical and laboratory-based studies from leading specialists in all lupus-related disciplines.

Invaluable reading, with extended coverage, lupus-related disciplines include:

- Rheumatology
- Dermatology
- Immunology
- Obstetrics
- Psychiatry
- Cardiovascular Research

Leading international specialists present their findings on Lupus, in one outstanding reference.

1.2 Article Types

Lupus is published fourteen times a year. The Editor will consider for publication all suitable papers dealing directly or indirectly with lupus or related diseases. The journal publishes both clinical and non-clinical research papers. Original research articles should be limited to no more than 4,000 words, 50 references and 6 tables and/or figures. They will be peer reviewed by two referees. In addition to original papers, the journal also publishes editorials, reports, and letters.

EDITORIALS

Editorials are solicited by the Editor but suggestions for such material will be very welcome.

GRAND ROUNDS CASES

The purpose of a grand rounds submission is to educate the reader about one or more facets related to the disease lupus or of an autoimmune disease which is related to lupus. A

clinicopathological conference can be submitted but this must have postmortem data and is usually a death conference or mortality conference.

Avoid extraneous material which has little bearing on the case at hand. The readers wish to learn about every facet of the case presented and not about other unrelated material.

The submitted case should contain:

Introduction - This should be no more than one or two short paragraphs and summarise what is about to be presented and the reasons why the case was chosen.

Case Presentation - This part contains a succinct narrative of the case itself. Figures, photographs and tables with data are welcome. Also encouraged are data on biopsies with illustrative materials if possible.

Discussion - The discussion should be a focused presentation of theory and/or pathogenetic data regarding the case.

Final Diagnosis - This should be only one sentence which gives the final diagnosis.

CONCISE REPORTS

These should be short investigative papers and reports organised in the same way as full-length manuscripts but which contain 2000 words or less, with no more than 3 figures or tables and up to 15 references.

CASE REPORTS

The Editor will consider for publication case reports that illustrate points not previously reported in the literature. They should not exceed two printed pages in length. The number of references should not exceed ten.

The number of case reports published in *Lupus* will be strictly limited.

LETTERS TO THE EDITOR

Letters to the Editor are encouraged. They may deal with material in published papers or they may raise new issues. Short clinical or laboratory observations may also be presented as Letters.

Letters must contain no more than 500 words, 10 references, 1 table and/or 1 illustration. An abstract is not required and letters should not be divided into sections. Instructions for references, tables and figures are the same as for full length articles.

SUPPLEMENTS

The journal welcomes the opportunity of publishing supplements to regular issues of significant symposia providing the material represents original work not previously published.

Sponsored symposia should be fully discussed with the Editor prior to agreement to publish. Faculty, subject matter and editorial content are all subject to the approval of the editorial office and the journal's integrity and reputation should in no way be compromised.

1.3 Writing your paper

The SAGE Author Gateway has some general advice and on how to get published, plus links to further resources.

1.3.1 Make your article discoverable

When writing up your paper, think about how you can make it discoverable. The title, keywords and abstract are key to ensuring readers find your article through search engines such as Google. For information and guidance on how best to title your article, write your

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2. Editorial policies

2.1 Peer review policy

The journal's policy is to obtain at least two independent reviews of each article. *Lupus* operates a conventional single-blind reviewing policy in which the reviewer's name is always concealed from the submitting author. Referees will be encouraged to provide substantive, constructive reviews that provide suggestions for improving the work and distinguish between mandatory and non-mandatory recommendations. All manuscripts accepted for publication are subject to editing for presentation, style and grammar. Any major redrafting is agreed with the author but the Editor's decision on the text is final.

As part of the submission process you will be asked to provide the names of peers who could be called upon to review your manuscript. Recommended reviewers should be experts in their fields and should be able to provide an objective assessment of the manuscript. Please be aware of any conflicts of interest when recommending reviewers. Examples of conflicts of interest include (but are not limited to) the below:

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- The reviewer should not have recently collaborated with any of the authors
- Reviewer nominees from the same institution as any of the authors are not permitted

Please note that the Editors are not obliged to invite any recommended/opposed reviewers to assess your manuscript.

2.2 Authorship

Papers should only be submitted for consideration once consent is given by all contributing authors. Those submitting papers should carefully check that all those whose work contributed to the paper are acknowledged as contributing authors.

The list of authors should include all those who can legitimately claim authorship. This is all those who:

1. Made a substantial contribution to the concept or design of the work; or acquisition, analysis or interpretation of data,
2. Drafted the article or revised it critically for important intellectual content,
3. Approved the version to be published,
4. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Authors should meet the conditions of all of the points above. When a large, multicentre group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship.

Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship, although all contributors who do not meet the criteria for authorship should be listed in the Acknowledgments section. Please refer to the International Committee of Medical Journal Editors (ICMJE) authorship guidelines for more information on authorship.

2.3 Acknowledgements

All contributors who do not meet the criteria for authorship should be listed in an Acknowledgements section. Examples of those who might be acknowledged include a

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Individuals who provided writing assistance, e.g. from a specialist communications company, do not qualify as authors and so should be included in the Acknowledgements section. Authors must disclose any writing assistance – including the individual's name, company and level of input – and identify the entity that paid for this assistance").

It is not necessary to disclose use of language polishing services.

Any acknowledgements should appear first at the end of your article prior to your Declaration of Conflicting Interests (if applicable), any notes and your References.

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2.5 Declaration of conflicting interests

It is the policy of *Lupus* to require a declaration of conflicting interests from all authors enabling a statement to be carried within the paginated pages of all published articles.

Please ensure that a 'Declaration of Conflicting Interests' statement is included at the end of your manuscript, after any acknowledgements and prior to the references. If no conflict exists, please state that 'The Author(s) declare(s) that there is no conflict of interest'.

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Medical research involving human subjects must be conducted according to the World Medical Association Declaration of Helsinki.

Submitted manuscripts should conform to the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, and all papers reporting animal and/or human studies must state in the methods section that the relevant Ethics Committee or Institutional Review Board provided (or waived) approval. Please ensure that you have provided the full name and institution of the review committee, in addition to the approval number.

For research articles, authors are also required to state in the methods section whether participants provided informed consent and whether the consent was written or verbal.

Information on informed consent to report individual cases or case series should be included in the manuscript text. A statement is required regarding whether written informed consent for patient information and images to be published was provided by the patient(s) or a legally authorized representative.

Please also refer to the ICMJE Recommendations for the Protection of Research Participants.

All research involving animals submitted for publication must be approved by an ethics committee with oversight of the facility in which the studies were conducted. The journal

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Lupus conforms to the ICMJE requirement that clinical trials are registered in a WHO-approved public trials registry at or before the time of first patient enrolment as a condition of consideration for publication. The trial registry name and URL, and registration number must be included at the end of the abstract.

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The relevant EQUATOR Network reporting guidelines should be followed depending on the type of study. For example, all randomized controlled trials submitted for publication should include a completed CONSORT flow chart as a cited figure and the completed CONSORT checklist should be uploaded with your submission as a supplementary file. Systematic reviews and meta-analyses should include the completed PRISMA flow chart as a cited figure and the completed PRISMA checklist should be uploaded with your submission as a supplementary file. The EQUATOR wizard can help you identify the appropriate guideline.

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SAGE acknowledges the importance of research data availability as an integral part of the research and verification process for academic journal articles.

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3. Publishing Policies

3.1 Publication ethics

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4. Preparing your manuscript for submission

Authors are asked to write their manuscripts in English. Spelling and phraseology should conform either to standard UK English or to standard American English and should be consistent throughout the paper.

The Summary should not exceed 200 words. It should be written in a style that conveys the essential message of the paper in abbreviated form.

The Introduction should assume that the reader is knowledgeable in the field and should therefore be as brief as possible.

In the Materials and methods section, methods that have been published in detail elsewhere should not be described in detail. SI units should be used throughout the text.

Tables and figures should be placed after references and not embedded within the text.

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The preferred format for your manuscript is Word. The text should be double-spaced throughout and with a minimum of 3cm for left and right hand margins and 5cm at head and foot. Text should be standard 10 or 12 point. LaTeX files are also accepted. Word and (La)Tex templates are available on the Manuscript Submission Guidelines page of our Author Gateway.

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Figures

Lettering should be planned for 50% reduction; text should be readable after reduction. Figures should be referred to as Figure 1, Figure 2 etc. Figures must be referred to specifically in the text of the paper.

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4.3 Supplementary material

This journal is able to host additional materials online (e.g. datasets, podcasts, videos, images etc) alongside the full-text of the article. For more information please refer to our guidelines on submitting supplementary files.

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It is important that references comply with the style of the journal. Exhaustive lists should be avoided. References should follow the Vancouver format, listed (double-spaced) in numerical order corresponding to the order of citation in the text.

All authors should be quoted for papers with up to six authors; for papers with more than six authors, the first three only should be quoted followed by et al.

No issue numbers should be quoted.

Abbreviations for titles of medical periodicals should conform to those used in the latest editions of Index Medicus and Current Contents. The first and last page numbers for each reference should be provided. Abstracts and letters must be identified as such.

Papers in press and papers already submitted for publication may be included in the list of references. No citation is required for work that is not yet submitted for publication.

Personal communications may be allocated a number and included in the list of references in the usual way or simply referred to in the text. Authors must obtain permission from the individual concerned to quote his or her unpublished work.

Examples of References:

Journal article:

1 Derksen RHWM, Bouma BN, Kater L. The association between the *Lupus* anticoagulant and cerebral infarction in systemic *Lupus* erythematosus. Scand J Rheumatol 1986; 15: 179-184.

Journal article, in press:

2 Mendonca LLF, Khamashta MA, Nelson-Piercy A, Hunt BJ, Hughes GRV. Nonsteroidal anti-inflammatory drugs as a possible cause for reversible infertility. Rheumatology (in press).

Journal article submitted for publication:

3 Khamashta MA, Cervera R, Asherson RA. Association of antibodies against phospholipids with heart valve disease in systemic *Lupus* erythematosus (submitted for publication).

Complete book:

4 Wallace DJ, Dubois EL. Dubois' *Lupus* Erythematosus. Lea & Febiger, 1987, p 51.

Chapter in book:

5 Christian CL. Etiologic hypotheses for systemic *Lupus* erythematosus. In: Lahita RG (ed)

Systemic *Lupus* Erythematosus. Wiley, 1987, 65-79.

Abstract:

6 Valesini G, Luan FL, Falco M. Clonal analysis of affinity purified anticardiolipin antibodies. Clin Exp Rheumatol 1988; 6: 214 (abstract 102).

Letter to the Editor:

7 Sills EM. Systemic *Lupus* erythematosus in a patient diagnosed as having Shulman disease. Arthritis Rheum 1988; 31: 694 (letter).

4.5 English language editing services

Authors seeking assistance with English language editing, translation, or figure and manuscript formatting to fit the journal's specifications should consider using SAGE Language Services. Visit SAGE Language Services on our Journal Author Gateway for further information.

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